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The quantification of carbonyl compounds produced from linoleic and linolenic acid hydroperoxides: thermal, metal ions and antioxidant effects

Bouali Saaidia
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THE QUANTIFICATION OF CARBONYL COMPOUNDS PRODUCED FROM LINOLEIC AND LINOLENIC ACID HYDROPEROXIDES: THERMAL, METAL IONS AND ANTIOXIDANT EFFECTS

Iowa State University PH.D. 1985

The quantification of carbonyl compounds produced from linoleic and linolenic acid hydroperoxides: Thermal, metal ions and antioxidant effects

by

Bouali Saaidia

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Approved:

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In Charge of Major Work

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INTRODUCTION

Lipid hydroperoxides are the initial products of autoxidation, and their decomposition results in a variety of carbonyl compounds that are largely responsible for the off-flavors and odors of deteriorated lipids. Even though there has been extensive investigation of the volatile products of autoxidizing triglycerides and other fatty esters, there is little information concerning the type of carbonyls and yields from the decomposition of pure lipid hydroperoxides.

To study the conversion of hydroperoxides to carbonyls, pure hydroperoxide isomers can be decomposed under anaerobic conditions. Recent work of this nature has been done at the temperatures of gas chromatographic injection ports (210°), but it is not clear how these hydroperoxides decompose under more realistic and less drastic conditions.

Recently in this laboratory, a rapid, quantitative method for carbonyl determination in oxidized oils was developed (97). This thesis is a report of the use of this method for the quantification of carbonyls resulting from the decomposition of cyclohexene hydroperoxide and fatty acid hydroperoxides. The effect of some antioxidants and metal ions on the scission products was also explored.

LITERATURE REVIEW

Fatty Acids Oxidation

The reaction of oxygen with the unsaturated fatty acids in lipids results in the formation of hydroperoxides as major initial products (24,71). Even though these hydroperoxides are tasteless and odorless, their degradation products are responsible for the off-flavors that characterize deteriorated lipid-containing foods.

Cyclohexene Hydroperoxide

Much information regarding the mechanism of autoxidation of olefinic compounds derived from fats and other mixtures has been obtained by studying the oxidation of simple, monounsaturated compounds such as cyclohexene. In 1928, Stephens (89) reported the isolation of a peroxide of cyclohexene which he obtained by treating cyclohexene with oxygen in daylight. He assumed, on the basis of the theories of oxidation accepted at that time, that the product was saturated. However, during the early 1940s, Farmer (20) and Farmer and Sundralingam (21) established that the product of Stephens was a hydroperoxide and that a double bond was present. It was also determined by Farmer's group that 1-methyl-1-cyclohexene and 1,2-dimethyl-1-cyclohexene behaved similarly when autoxidized. To this group is due the major credit for developing the hydroperoxide hypothesis of autoxidation, especially in its application to fatty acids, and for substantiating it with convincing experimental evidence.

Autoxidation and photo-oxidation

The oxidation of fatty acids may proceed by a free radical mechanism with atmospheric oxygen or by photo-oxidation involving singlet oxygen. Generally accepted mechanisms for lipid autoxidation have been reviewed recently (25,26,27,78). Autoxidation is conceived as a free radical chain reaction that can be described in terms of initiation, propagation, and termination processes.

Initiation : Formation of free radicals

$$RH \longrightarrow R\cdot + H\cdot$$

In this stage, an unsaturated hydrocarbon loses a hydrogen to form free radicals. This reaction may take place by direct thermal dissociation (thermolysis), by hydroperoxide decomposition, by metal catalysis, and by exposure to light (photolysis), with or without the intervention of photosensitizers.

Propagation : Free radical chain reaction

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$

During propagation, the chain reaction forms peroxy radicals (ROO), hydroperoxides (ROOH), and new hydrocarbon radicals (R).

Termination : Formation of nonradical products

$$R\cdot + R\cdot \longrightarrow RR$$

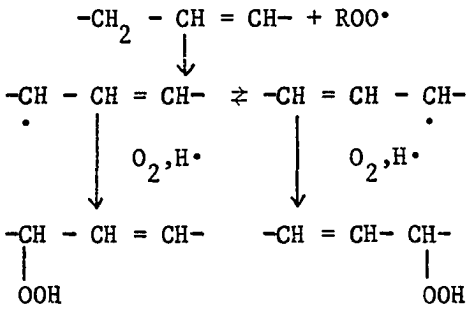
$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$$

$$RO\cdot + R\cdot \longrightarrow ROR$$

$$ROO\cdot + R\cdot \longrightarrow ROOR$$

$$2RO\cdot + 2ROO\cdot \longrightarrow 2ROOR + O_2$$

Farmer et al. (20,23) showed that the hydrogen extracted from RH was adjacent or allylic to the double bond. The susceptibility of unsaturated fatty acids to autoxidation varies according to the ability of their allylic hydrogens. The attack on the "α-methylene group" leads to the formation of allylic radicals that react with oxygen to form allylic hydroperoxides:



The reaction of singlet oxygen proceeds by a different mechanism from free radical autoxidation (35,43). The activated oxygen involved in this reaction is produced from the more usual triplet form of this element by interaction of light and a sensitizer such as chlorophyll, rose bengal, or methylene blue. The reaction proceeds by insertion of oxygen at either end carbon of a double bond, which is shifted to yield an allylic hydroperoxide with the double bond in the "trans" configuration.

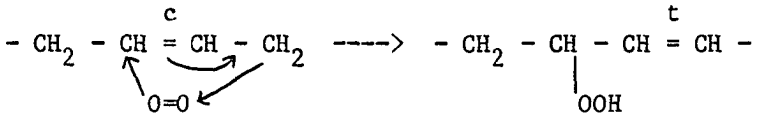


Photo-oxidation occurs at a much faster rate than autoxidation and produces some different hydroperoxide isomers (43).

Mechanism of action of antioxidants

Antioxidants are substances that have been found to be effective in retarding oxidation of unsaturated acids in fats and oils, thus preventing development of oxidized flavor defects. They are usually thought to function as free radical acceptors, thus breaking the free radical chain reaction that forms hydroperoxides:

$$RH \xrightarrow{-H\cdot} R\cdot \xrightarrow{O_2} ROO\cdot \xrightarrow{RH} ROOH + R\cdot$$

$$AH \xrightarrow[ROO\cdot]{R\cdot} A\cdot + \begin{matrix} RH \\ ROOH \end{matrix}$$

Phenolic antioxidant — Relatively stable antioxidant

There is abundant literature (69,70,88,90) about the mechanisms of antioxidant action and the effectiveness of various antioxidants, but information about their effects on hydroperoxide scission is very meager if not totally absent.

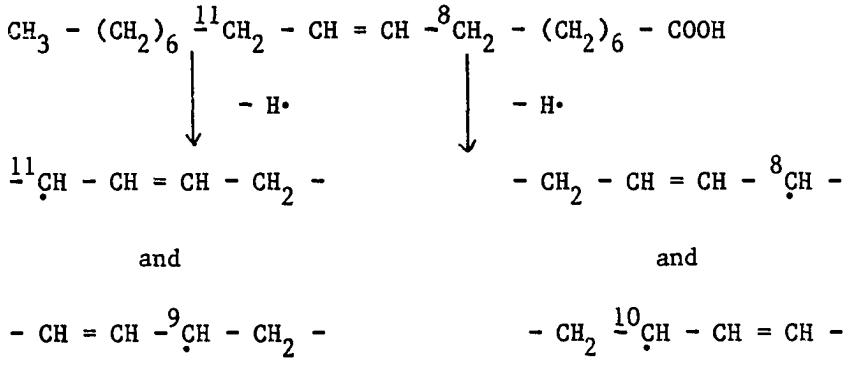
Fatty Acid Hydroperoxides

Hydroperoxides from oleic acid

Farmer and Sutton (22) isolated methyl oleate peroxides and showed that these somewhat impure peroxides consisted mainly of monohydroperoxide but also contained a little dihydroperoxide together with some peroxide transformation product. The first structural characterization of oleate hydroperoxides was carried out by Ross et al. (82). They found the four possible isomers predicted by Farmer's theory and reported shift of the double bond in the autoxidation of methyl oleate. Piretti et al. (80) reported obtaining four isomers in mixtures of hydroperoxides from

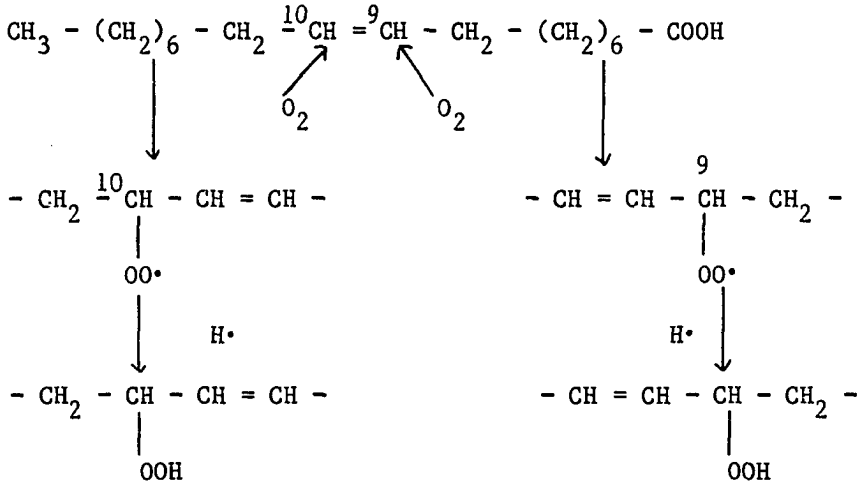
methyloleate autoxidation at different temperatures. Different structural characterization schemes have been used to determine the isomeric distribution of oleate hydroperoxide and the results of different workers were summarized by Frankel et al. (29).

According to Frankel (24), the most accepted mechanism for oleate autoxidation involves hydrogen abstraction at carbon 8 and 11 with the formation of four resonance hybrid free radicals:



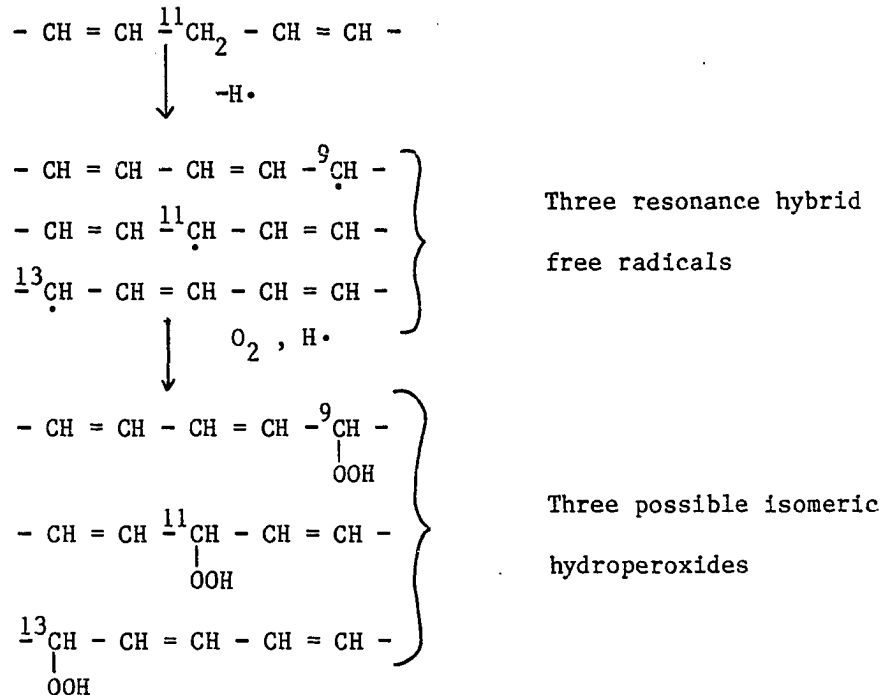
These radicals react with oxygen to produce a mixture of 8-, 9-, 10-, and 11-peroxy radicals leading to the formation of four isomeric hydroperoxides. Although these isomers are expected to form in equal amounts, a wide variation in their relative distribution has been reported by different workers (29). Recent studies (29,74) showed small but consistently higher concentrations of the 8- and 11-hydroperoxides than the 9- and 10-. These differences were explained (29) on the basis of an allylic isomerization of the hydroperoxide isomers.

The photosensitized oxidation of methyl oleate was studied by Chan (10) who used riboflavin as a sensitizer. The hydroperoxide mixture was found to contain only the 9- and 10- positional isomers. Their mechanism of formation is as follows:



Hydroperoxides from linoleic acid

Autoxidation According to the mechanism of reaction originally proposed by Bolland and Koch (9), the abstraction of a hydrogen on the reactive doubly allylic carbon-11 produces three resonance hybrid free radicals that lead to the formation of three isomeric hydroperoxides:



Many studies have been carried out to find the position of the hydroperoxide group in linoleate hydroperoxides. Bergström (6), after hydrogenating autoxidized methyl linoleate, isolated 9- and 13-hydroxystearates but not 11-hydroxystearate. His results, therefore, left it an open question whether any 11-hydroperoxide or other unconjugated hydroperoxide is formed to any appreciable extent during the autoxidation of methyl linoleate. From kinetic studies on the autoxidation of ethyl linoleate, Bolland and Gee (8) concluded that there would be little probability of the formation of appreciable amounts of unconjugated 11-hydroperoxide. Other workers (7,45,86) also reported the absence of 11-hydroperoxide, but Khan et al. (62) and Lundberg et al. (72) found some 11-hydroperoxide in the autoxidation of methyl linoleate.

Chan and Levett (11), by high performance liquid chromatography (HPLC) and absorption chromatography could separate and identify four major isomers as 9- and 13-positional isomers having the trans-trans and cis-trans configuration. Frankel et al. (30) used a computerized GC-MS method to analyze a large number of samples of methyl linoleate obtained at different peroxide values (93 to 3150) and autoxidation temperatures (40°C to 80°C). All the samples showed equal amounts of 9- and 13-hydroxy isomers.

Recently, Haslbeck and Grosh (51) showed that when phenyl linoleate is autoxidized, in addition to the major products (9- and 13-hydroperoxides), four other hydroperoxides (8-, 10-, 12-, and 14-hydroperoxides) are produced in small quantities (4%).

Photo-oxidation Photo-oxidation of linoleate forms a mixture of 9-, 10-, 12-, and 13-hydroperoxides (16). Frankel et al. (28) determined the relative amounts of these isomers: 32% 9-, 17% each of 10- and 12-, and 35% 13-hydroperoxides.

Hydroperoxides from linolenate

The mechanism for linolenate autoxidation is based on that of linoleate. Hydrogen abstraction on the two active methylenes on carbon 11 and carbon-14 produces four resonance hybrid free radicals on carbon 9, 12, 13, and 16, leading to the formation of the four corresponding hydroperoxides. Chan and Levett (12) analyzed the mixture of methyl hydroxylinolenates obtained by reduction of the hydroperoxides. They found four positional isomers with the hydroxyl group at positions 9, 12, 13, and 16. Furthermore, the relative proportions of the four positional isomers are not equal but favor the 9- and 16-isomers by a ratio of over 3 to 1. The authors concluded that the autoxidation of methyl linolenate is regioselective in favoring the formation of the "outer" isomers: the 9- and 16-hydroperoxides. Frankel et al. (31) also found that the proportion of 9- and 16-hydroperoxides was significantly higher (75-81%) than the 12- and 13-hydroperoxides (18-25%). They explained these differences by the tendency of the 12- and 13-hydroperoxides to form cyclic peroxides, cyclic peroxide-hydroperoxides, and prostaglandin-like endoperoxides.

Photosensitized oxidation of linolenate produces, in addition to the four isomers produced by autoxidation, the 10- and 15-hydroperoxides (16,74,94).

Table 1 summarizes the hydroperoxides formed by autoxidation and photo-oxidation of oleate, linoleate, and linolenate.

Fatty acid hydroperoxides formed by plant lipoxygenases

Lipoxygenases catalyze the oxidation by molecular oxygen of linoleic and other polyunsaturated fatty acids to hydroperoxides. The reaction mechanism and specificity of plant lipoxygenases were reviewed by Veldink et al. (96).

The ratio of 13- to 9-hydroperoxides formed from linoleic acid with soybean lipoxygenases has been variously quoted as 7:3 (46), 4:1 (99), 23:2 (47), and 100:0 (17).

Galliard and Phillips (37) partially purified a lipoxygenase from potato tubers that converted linoleic acid almost exclusively (95%) into 9-D-hydroperoxyoctadeca-trans-10, cis-12-dienoic acid. The 13-hydroperoxide isomer was formed only as a minor product (5%). A crude potato extract gave the same isomer ratio. Linolenic acid was an equally effective subtrate, and was also oxygenated specifically at the 9-position.

Hydroperoxide Decomposition Products

Scission of hydroperoxides

Secondary degradation products of lipid oxidation are formed largely from the decomposition of hydroperoxides, which involves a very complicated set of reaction pathways. The early schemes advanced by Bell et al. (5) for the general decomposition of peroxides to scission products are

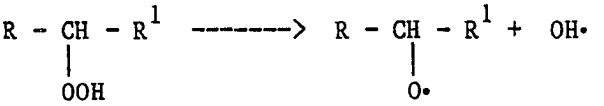
Table 1. Hydroperoxides formed by autoxidation and photo-oxidation of oleate, linoleate, and linolenate

Fatty acid	Isomeric hydroperoxides	Hydroperoxide position[a]
Oleic	$CH_3-(CH_2)_6-CH_2-CH = CH-CH_2-(CH_2)_6-COOH$	
	$CH_3-(CH_2)_6-CH_2-CH = CH-\underset{\displaystyle OOH}{CH}-(CH_2)_6-COOH$	8 (A)
	$CH_3-(CH_2)_6-CH = CH-\underset{\displaystyle OOH}{CH}-(CH_2)_7-COOH$	9 (A,P)
	$CH_3-(CH_2)_6-(CH_2)-\underset{\displaystyle OOH}{CH}-CH = CH-(CH_2)_6-COOH$	10 (A,P)
	$CH_3-(CH_2)_6-\underset{\displaystyle OOH}{CH}-CH = CH-CH_2-(CH_2)_6-COOH$	11 (A)
Linoleic	$CH_3-(CH_2)_4-CH = CH-CH_2-CH = CH-(CH_2)_7-COOH$	
	$CH_3-(CH_2)_4-CH = CH-CH = CH-\underset{\displaystyle OOH}{CH}-(CH_2)_7-COOH$	9 (A,P)
	$CH_3-(CH_2)_4-CH = CH-CH_2-\underset{\displaystyle OOH}{CH}-CH = CH-(CH_2)_6-COOH$	10 (P)

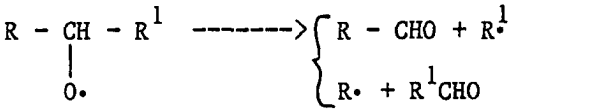
	$CH_3-(CH_2)_3-CH = CH-\underset{\underset{OOH}{|}}{CH}-CH_2-CH = CH-(CH_2)_7-COOH$	12 (P)
	$CH_3-(CH_2)_4-\underset{\underset{OOH}{|}}{CH}-CH = CH-CH = CH-(CH_2)_7-COOH$	13 (A,P)
Linolenic	$CH_3-CH_2-CH = CH-CH_2-CH = CH-CH_2-CH = CH-(CH_2)_7-COOH$	
	$CH_3-CH_2-CH = CH-CH_2-CH = CH-CH = CH-\underset{\underset{OOH}{|}}{CH}-(CH_2)_7-COOH$	9 (A,P)
	$CH_3-CH_2-CH = CH-CH_2-CH = CH-CH_2-\underset{\underset{OOH}{|}}{CH}-CH = CH-(CH_2)_6-COOH$	10 (P)
	$CH_3-CH_2-CH = CH-CH = CH-\underset{\underset{OOH}{|}}{CH}-CH_2-CH = CH-(CH_2)_7-COOH$	12 (A,P)
	$CH_3-CH_2-CH = CH-CH_2-\underset{\underset{OOH}{|}}{CH}-CH = CH-CH = CH-(CH_2)_7-COOH$	13 (A,P)
	$CH_3-CH = CH-\underset{\underset{OOH}{|}}{CH}-CH_2-CH = CH-CH_2-CH = CH-(CH_2)_7-COOH$	15 (P)
	$CH_3-CH_2-\underset{\underset{OOH}{|}}{CH}-CH = CH-CH = CH-CH_2-CH = CH-(CH_2)_7-COOH$	16 (A,P)

[a] A: Formed by autoxidation only. P: Formed by photo-oxidation only. A,P: Formed by autoxidation and photo-oxidation.

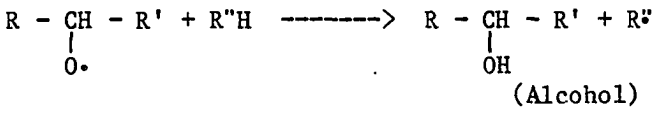
still generally accepted today. The first step is visualized as decomposition to the alkoxy- and hydroxy-free radicals:



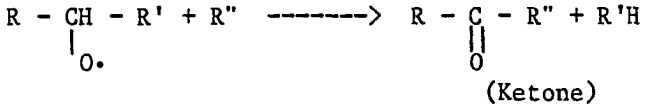
The secondary alkoxy radical may then cleave on either side of the radical to yield an aldehyde and a new alkyl-type radical (β-scission):



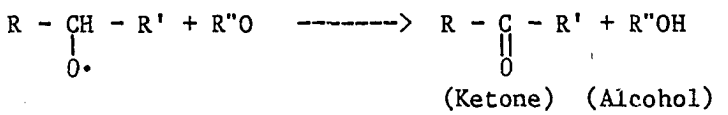
It can also react as in the following reactions:



(Alcohol)



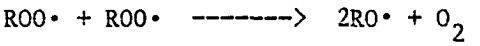
(Ketone)



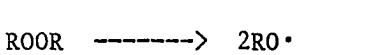
(Ketone) (Alcohol)

Alkoxy radicals may also be formed by:

--interaction of peroxy radicals:



--homolytic cleavage of a peroxide:



The thermal homolysis of the O-O bond is complicated by induced decomposition in which radicals attack hydroperoxides (52):

$$RO\cdot + ROOH \longrightarrow ROH + ROO\cdot$$

$$2ROO\cdot \longrightarrow 2RO\cdot + O_2$$

Other routes for radical production include:

--bimolecular decomposition of hydroperoxides:

$$2ROOH \longrightarrow RO\cdot + ROO\cdot + H_2O$$

$$ROO\cdot + RH \longrightarrow ROOH + R\cdot$$

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

--metal-catalyzed decomposition.

Transition metal ions and metallaproteins effectively catalyze homolytic reactions of hydroperoxides as outlined by Ingold (56):

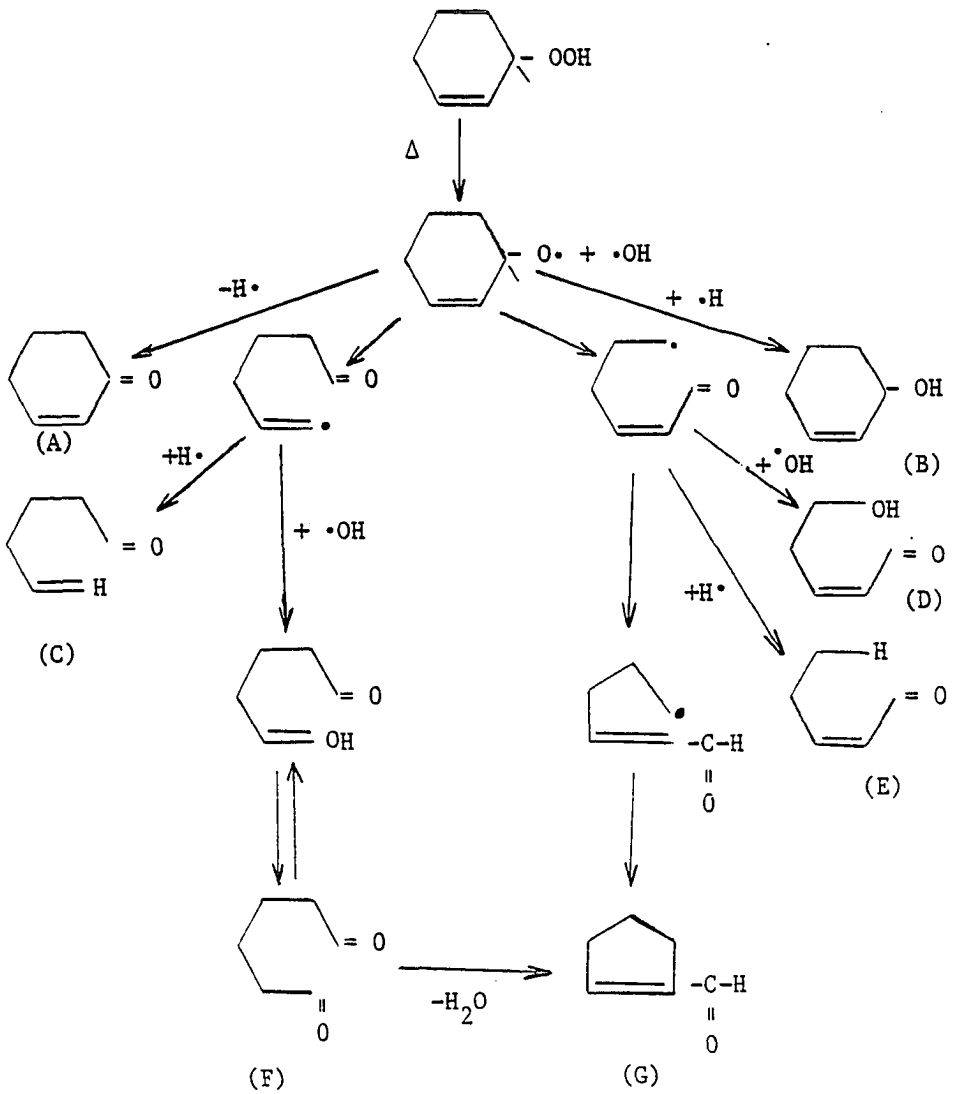
$$M^{n+} + ROOH \longrightarrow M^{(n+1)+} + RO\cdot + OH^-\ ,$$

$$M^{(n+1)+} + ROOH \longrightarrow M^{n+} + ROO\cdot + H^+\ ,\ \text{or}$$

$$2ROOH \longrightarrow RO\cdot + ROO\cdot + H_2O\ .$$

Decomposition of cyclohexene hydroperoxide

Homolytic cleavage products The products expected from decomposition of cyclohexene hydroperoxide and their route of formation are presented in the set of reactions on the following page.



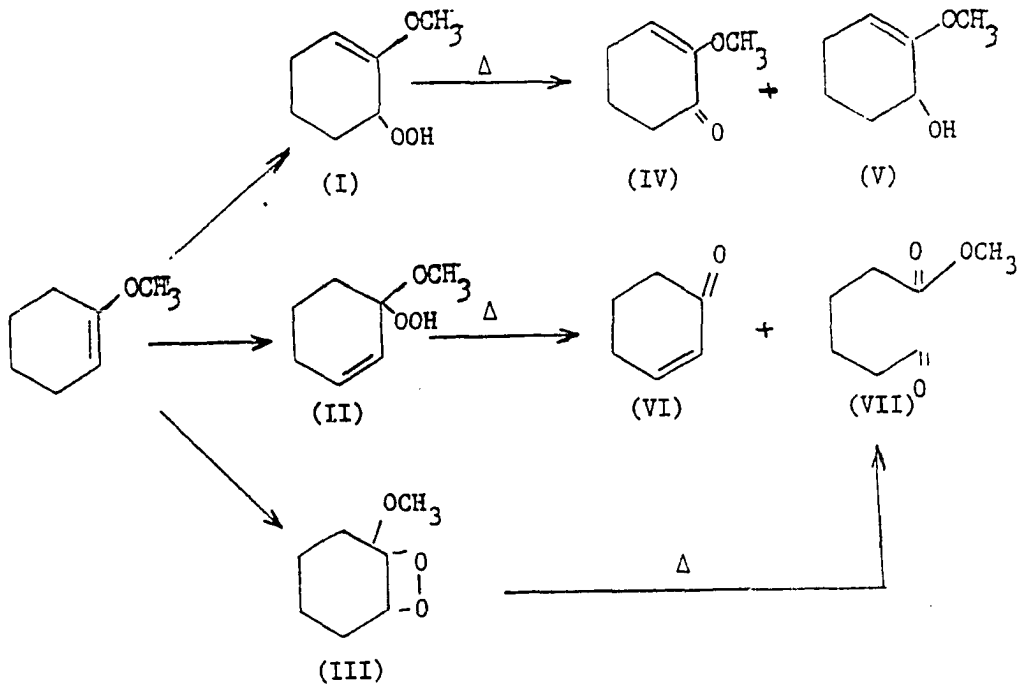
A: 2-cyclohexenone
B: 2-cyclohexenol
C: 5-hexenal
D: 6-hydroxy-2-hexenal
E: 2-hexenal
F: 1,6-hexanedial (adipaldehyde)
G: cyclopentene carboxaldehyde

As reported in the early investigations on the thermal decomposition of cyclohexene hydroperoxides, 2-cyclohexenol and/or 2-cyclohexenone were the major volatiles formed. Farmer and Sundralingam (21) isolated 2-cyclohexenol as the principal volatile product from the decomposition of the hydroperoxide at 100°C. They also reported that when the hydroperoxide was gradually heated in an open test tube, standing in an oil bath, very vigorous decomposition set in at about 120°C, steam and volatile vapors being evolved, the latter containing cyclopentene carboxaldehyde. Bateman and Hughes (4) decomposed cyclohexene hydroperoxide in benzene under vacuum at 100°C for 42 hours and identified 2-cyclohexenone and 2-cyclohexenol as decomposition products. Their molar yields were 30% and 13%, respectively.

Compounds C, D, E, F, G are β-scission products of the alkoxy free radical and their quantification could be a measurement of the extent of this reaction. Cyclopentene carboxaldehyde would be the predominant carbonyl in this group since there are at least two pathways for its formation.

An interesting insight into the relative importance of the β-cleavage reaction to produce carbonyls was gained from decomposition of the oxidation products of 1-methoxycyclohexene (3) as outlined on page 16. Photooxidation of 1-methoxycyclohexene produces hydroperoxides (I) and (II) and a dioxetane (III) as primary products. 3-Hydroperoxy-2-methoxycyclohexene (I) is thermolyzed in the VPC injector port to the corresponding allylic ketone (IV) and alcohol (V). 3-Hydroperoxy-3-methoxycyclohexene (II) is thermolyzed to cyclohexenone (VI) and

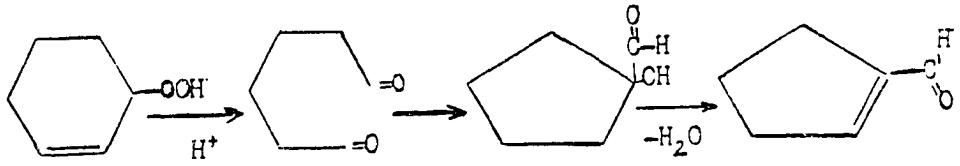
Hock-cleavage product, dione (VII). The latter is also the dioxetane cleavage product:



Seemingly in the case of cyclic secondary allylic hydroperoxides, and at high temperatures, β-cleavage does not occur.

Heterolytic decomposition of cyclohexene hydroperoxide

Under acidic conditions, cyclohexene hydroperoxide decomposes heterolytically to form compounds (F) and (G), a reaction referred to as Hock-cleavage, after Hock and Schrader (55) who first reported in 1936 that cyclohexene hydroperoxide, when allowed to stand with sulfuric acid at 35-40°C, gave cyclopentene carboxaldehyde in about 20% yield:

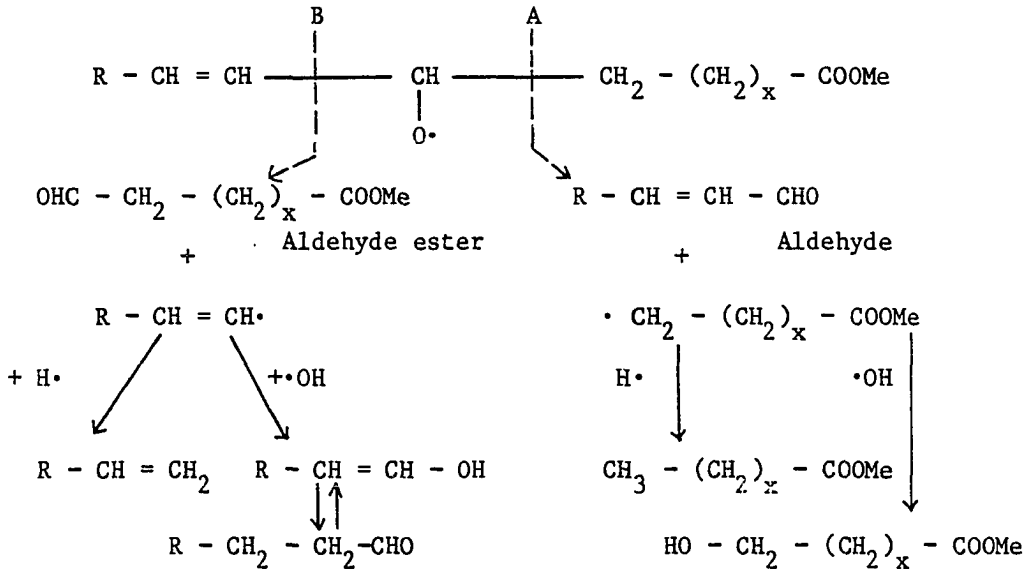


F G

Kharash and Burt (63) decomposed cyclohexene hydroperoxide in the presence of glacial acetic acid and perchloric acid. The major products reported were cyclopentene carboxaldehyde (G, 39% yield) and cyclohexenyl acetate (38%). Adipaldehyde was also formed (6%).

Decomposition of fatty acid hydroperoxides

The breakdown of secondary alkoxy radicals leading to the β-scission recognized for alkyl hydroperoxides has been generally accepted and authenticated for the allylic system of fatty ester hydroperoxides (24,61). The carbon-carbon cleavages on both sides of the alkoxy radical (A, B) leading to aldehydes and aldehyde esters can be represented as follows:



A survey of the aldehydes which would form according to this scheme from the decomposition of oleate, linoleate, and linolenate hydroperoxides is presented in Table 2.

Considerable effort has been devoted to isolating, identifying, and sometimes to quantifying the carbonyls resulting from fatty acid hydroperoxides. The hydroperoxide dismutation theory can be used to explain the occurrence of most of the decomposition products. However, reaction variations have been suggested by numerous authors in speculating about the mechanism of formation of specific or unexpected compounds found in oxidized fatty acid esters or pure fatty acid hydroperoxides. These speculations were, in general, based on the special conditions used in the experiment, such as substrates or separation and analytical techniques used.

Oleate hydroperoxides decomposition products Swift et al. (91) decomposed methyl oleate hydroperoxide by immersing it in an oil bath

Table 2. Aldehydes expected from the decomposition of fatty acid hydroperoxides

Fatty acid	Alkoxy radical involved	Cleavage side	Aldehydes
Oleic	$CH_3-(CH_2)_7-CH = CH-(CH_2)_7-COOH$		
	$CH_3(CH_2)_7-CH = CH \overset{B}{\dagger} \underset{8}{CH(O\cdot)} \overset{A}{\dagger} (CH_2)_6-COOH$	A: B:	2-Undecenal Decanal
	$CH_3(CH_2)_6-CH = CH \overset{B}{\dagger} \underset{9}{CH(O\cdot)} \overset{A}{\dagger} (CH_2)_7-COOH$	A: B:	2-Decenal Nonanal
	$CH_3(CH_2)_7 \overset{B}{\dagger} \underset{10}{CH(O\cdot)} \overset{A}{\dagger} CH = CH-(CH_2)_6-COOH$	A:	Nonanal
	$CH_3(CH_2)_6 \overset{B}{\dagger} CH(O\cdot) \overset{A}{\dagger} CH = CH-(CH_2)_7-COOH$	A:	Octanal
Linoleic	$CH_3(CH_2)_4-CH = CH-CH-CH = CH-(CH_2)_7-COOH$		
	$CH_3(CH_2)_4-CH = CH-CH = CH \overset{B}{\dagger} \underset{9}{CH(O\cdot)} \overset{A}{\dagger} (CH_2)_7-COOH$	A: B:	2,4-Decadienal 3-Nonenal
	$CH_3(CH_2)_4 \overset{B}{\dagger} \underset{13}{CH(O\cdot)} \overset{A}{\dagger} CH = CH-CH = CH-(CH_2)_7-COOH$	A:	Hexanal
	$CH_3(CH_2)_4-CH = CH-CH_2 \overset{B}{\dagger} \underset{10}{CH(O\cdot)} \overset{A}{\dagger} CH = CH(CH_2)_6-COOH$[a]	A:	3-Nonenal

	$CH_3(CH_2)_3-CH = CH \overset{B}{\underset{12}{\dagger}} CH(O\cdot) \overset{A}{\dagger} CH_2-CH = CH(CH_2)_7-COOH$[a]	A:	2-Heptenal
		B:	Hexanal
Linolenic	$CH_3CH_2-CH = CH-CH-CH = CH-CH-CH = CH-(CH_2)_7-COOH$		
	$CH_3CH_2-CH = CH-CH_2-CH = CH-CH = CH \overset{B}{\underset{9}{\dagger}} CH(O\ ) \overset{A}{\dagger} (CH_2)_7-COOH$	A:	2,4,7-Decatrienal
		B:	3,6-Nonadienal
	$CH_3-CH_2-CH = CH-CH = CH \overset{B}{\underset{12}{\dagger}} CH(O\cdot) \overset{A}{\dagger} CH_2-CH = CH-(CH_2)_7-COOH$	A:	2,4-Heptadienal
		B:	3-Hexenal
	$CH_3-CH_2-CH = CH-CH_2 \overset{B}{\underset{13}{\dagger}} CH(O\cdot) \overset{A}{\dagger} CH = CH-CH = CH-(CH_2)_7-COOH$	A:	3-Hexenal
		B:	Pentenal
	$CH_3-CH_2 \overset{B}{\underset{16}{\dagger}} CH(O\cdot) \overset{A}{\dagger} CH = CH-CH = CH-CH_2-CH = CH-(CH_2)_7-COOH$	A:	Propanal
	$CH_3CH_2-CH = CH-CH_2-CH = CH-CH_2 \overset{B}{\underset{10}{\dagger}} CH(O\cdot) \overset{A}{\dagger} CH = CH-(CH_2)_6-COOH$[a]	A:	3,6-Nonadienal
	$CH_3CH = CH \overset{B}{\underset{15}{\dagger}} CH(O\cdot) \overset{A}{\dagger} CH_2-CH = CH-CH_2-CH = CH-(CH_2)_7-COOH$[a]	A:	2-Butenal
		B:	Propanal

[a]From photo-oxidation.

heated to 150°C. They isolated different distillate fractions from the decomposition mixture and prepared their 2,4-dinitrophenylhydrazones (DNPH). Their results indicated that one of the decomposition reactions is fission to produce α, β-unsaturated carbonyl compounds, one of which is 2-undecenal.

Badings (2) cited a study done by Horikx and Schogt in 1959. Horikx and Schogt studied the carbonyl compounds formed in the initial phase of autoxidation of methyl oleate and triolein. They identified the saturated aldehydes of chain lengths C_5 to C_{11}, and the C_6 to C_{12} 2-enals. The formation of C_8 and C_9 n-aldehydes and C_{10} and C_{11} 2-enals was in line with the theory of decomposition of four isomeric hydroperoxides of oleic acid.

Gaddis et al. (36) quantitatively estimated the steam volatile monocarbonyl compounds in mildly autoxidized esters of oleic, linoleic, and linolenic acids and animal and vegetable fats. The major aldehydes isolated in oleate were those that might be expected from the scission of the reported four monomeric hydroperoxide isomers. 2-Undecenal, 2-decenal, n-nonanal, and n-octanal were the major compounds but were not individually equal. They also found that higher temperatures seem to increase the proportions of the major aldehydes that were unsaturated (2-undecenal and 2-decenal).

Unsaturated aldehydes were not present in the volatile compounds isolated by Loury (68) from the autoxidation of oleate. He claimed that this might be caused by either the severe conditions of autoxidation used,

or the fact that these aldehydes are engaged in other combinations during the primary stages of autoxidation.

Kimoto and Gaddis (65) converted the monocarbonyl compounds formed from the decomposition of autoxidized triolein into their 2,4-dinitrophenylhydrazones and analyzed them by thin layer chromatography and paper chromatography. Special conditions such as the presence of acid and metal catalysts were investigated. In the decomposition of autoxidized triolein with acid-washed fuller's earth, alkanals were the only monocarbonyl products found, whereas when metal catalysts or heat were used to decompose the peroxides, 2-alkenals were the primary products. From the differences in distribution of monocarbonyl products, the authors concluded that the scission of the hydroperoxides is a selective process depending upon the conditions of decomposition: heterolytic (ionic) cleavage catalyzed by strong acids and homolytic (radical) cleavage with heat or metal catalysts.

Selke et al. (84) thermally decomposed pure methyl oleate hydroperoxides in the injector port (210°C) of a gas chromatograph attached to a computerized mass-spectrometer (GC-MS). Carbonyls identified were those expected from the well-recognized mechanism of carbon-carbon scission on either side of the alkoxy radical intermediate produced from hydroperoxides, except for heptanal and 2-nonenal. The yields of A and B cleavage products from the 8-, 9-, 10-, and 11-hydroperoxides were checked and found in good agreement. The presence of great amounts of octanal, nonanal, and decanal indicated that cleavage occurring between the alkoxy-free radical and the allylic unsaturation seemed to be favored kinetically

with oleate hydroperoxides at high temperatures. Using the same GC-MS technique, Frankel et al. (32) compared the thermal decomposition products of pure oleate hydroperoxides from autoxidation and photosensitized oxidation. Both kinds of hydroperoxides formed the same major volatile products. The great relative value for 2-undecenal found in the photosensitized sample was explained by the significant isomerization of the hydroperoxides mixture observed under the conditions of thermal decomposition.

Linoleate hydroperoxides decomposition products The decomposition products of linoleate hydroperoxides have received the most attention since linoleate is the major unsaturated fatty acid in most vegetable oils.

Swift et al. (92) collected the steam-volatile products from autoxidized cottonseed oil and identified n-hexanal, 2,4-decadienal, and 2-octenal. Badings (1) also reported the formation of these carbonyls from a limited autoxidation of ammonium linoleate. The hydroperoxide scission theory was advanced to explain the formation of hexanal and 2,4-decadienal as dismutation products of the 9- and 13-hydroperoxides. The formation of 2-octenal was attributed to the decomposition of a non-conjugated 11-hydroperoxide. However, this hydroperoxide has never been detected under a wide range of autoxidation conditions (25,74).

Gaddis et al. (36) determined the carbonyl compounds in oxidized ethyl linoleate and found n-hexanal, 2,4-decadienal as major products. 2-Heptenal, 2-octenal, and 2-nonenal were also present as a minor group with the predominance of 2-heptenal. Other workers (2,19,85) also

reported 2-heptenal in autoxidized linoleate esters and fats containing linoleate. When pure hydroperoxides from autoxidized and photosensitized oxidized linoleate were compared (32), 2-heptenal was detected almost exclusively in the photo-oxidized sample. Therefore, the origin of 2-heptenal would be the 12-hydroperoxide isomer.

It is well-established that the 9- and 13-hydroperoxide isomers form in equal amounts in linoleate autoxidation. Hence, 2,4-decadienal and n-hexanal are expected to form equally. However, early reports (64) showed a preferential formation of hexanal at moderate temperatures and 2,4-decadienal at higher temperatures. Swoboda and Lea (93) explained this effect of temperature by further selective oxidation of 2,4-decadienal. Kimoto and Gaddis (64) advanced another explanation. They compared the carbonyls obtained from lard under different situations (mild temperatures, heat, Cu^{++}, acid, etc.) and explained the low amount of 2,4-decadienal obtained under the mild conditions by a selective course of scission of the hydroperoxides: at moderate temperatures, the point of scission tends to be at the carbon-carbon bond between the peroxide group and a double bond, therefore, favoring the formation of hexanal. At higher temperatures and under stress (heat, acid, Cu^{++}), scission also takes place at the 9-8 carbons to yield 2,4-decadienal.

Individual studies (14) of the decomposition of the positional isomers of linoleate hydroperoxides (9- and 13-) revealed that, at temperatures above 100°C and in the absence of oxygen, both the 9- and 13-isomers gave rise to hexanal and 2,4-decadienal. To explain this, it was assumed (14) that rearrangement of the hydroperoxy group is involved

in the homolytic cleavage of the hydroperoxides. Chan et al. (13,15) had shown previously that both the 9- and 13-isomers of linoleate hydroperoxide undergo isomerization readily at low temperatures. However, at ambient temperature and in the presence of oxygen and ascorbic acid, hexanal resulted as the major volatile carbonyl compound in addition to traces of 2,4-decadienal from both the 9- and 13-hydroperoxides (42). Hexanal was also found (41) to be the main component (70%) of the aldehyde fraction when a mixture of linoleic acid hydroperoxide isomers (75% 13-OOH, 25% 9-OOH) was decomposed at ambient temperature in the presence of ferrous ions or ascorbic acid.

Linolenate hydroperoxide decomposition products Most of the products expected from the scission scheme of linolenate hydroperoxides have been reported. Kawahara et al. (60) identified acetaldehyde, 2-pentenal, propanal, and hexene-3-dial-1,6 as carbonyl compounds of autoxidizing methyl linolenate. The major carbonyl compounds identified by Gaddis et al. (36) were propanal and 2,4-heptadienal. They also reported the presence of butanal, 2-butenal, 2-pentenal, and 2-hexenal and found that heating had the effect of making 2,4-heptadienal and 2,4-nondienal the predominant compounds.

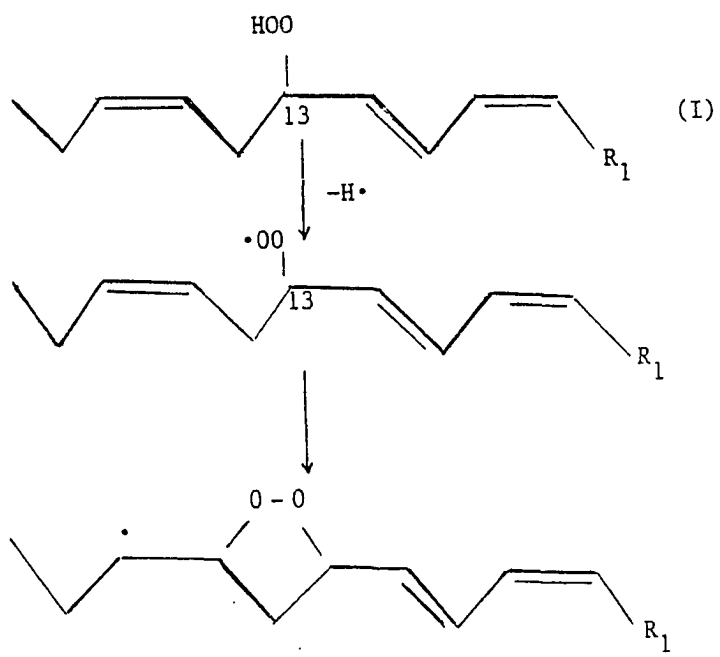
Kimoto and Gaddis (65) reported that thermal- and metal-catalyzed decomposition of autoxidized methyl linolenate resulted in the formation primarily of propanal and 2,4-heptadienal, whereas with acid washed fuller's earth, the primary products were propanal, 2-hexenal, and what was believed to be 2,6-nonadienal.

Hammond and Hill (50) found 2-trans-6-cis-nonadienal as a decomposition product of autoxidized trilinolenin. Seals and Hammond (83) identified cis-4-heptenal and 2,4-pentadienal in autoxidized linseed oil and proposed mechanisms for their formation.

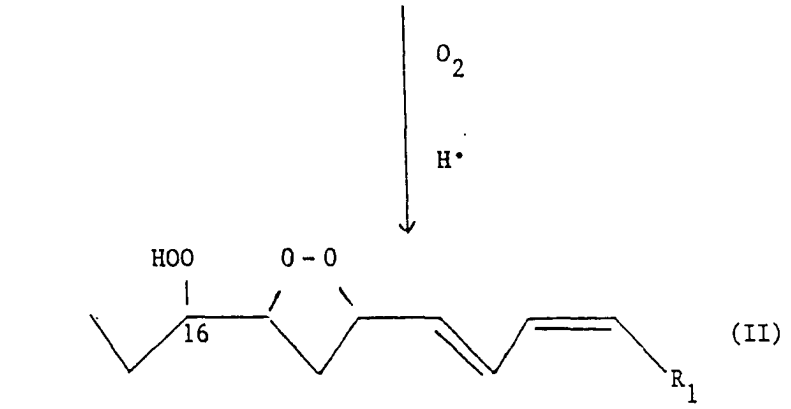
In their extensive investigation of the volatile thermal decomposition products of hydroperoxides obtained by autoxidation and photosensitized oxidation of fatty acid methyl esters, Frankel et al. (32) reported the formation of acrolein, propanal, 3-hexenal, 2,4-heptadienal, and 2,4-decatrienal as major volatile carbonyls from linolenate hydroperoxides obtained by autoxidation. Thermal decomposition of the hydroperoxide from photosensitized oxidized methyl linolenate produced many of the same volatiles as did the corresponding hydroperoxides for autoxidized linolenate, but in different proportion. Peers et al. (79) prepared purified single isomers of methyl linolenate hydroperoxide (9-, 12-, 13-, and 16-hydroperoxides) and examined the thermal decomposition products under the same conditions used by Chan et al. (14). They reported many of the volatiles found by Frankel et al. (32). The predominant characteristic compounds were 2,4,7-decatrienals from the 9-hydroperoxide, 2,4-heptadienals from the 12-hydroperoxide, and methyl 13-oxotrideca-9,11-dienoates from the 13-hydroperoxide. The 16-hydroperoxide produced no detectable characteristic volatiles but did produce trace amounts of 2,4-heptadienals and 4,5-epoxyheptadienal. The authors also showed that under their thermal decomposition very little, if any, isomerization of the individual hydroperoxides had occurred before fragmentation, which was not the case with the 9- and 13-hydroperoxide isomers of linoleate (14).

Secondary oxidation products

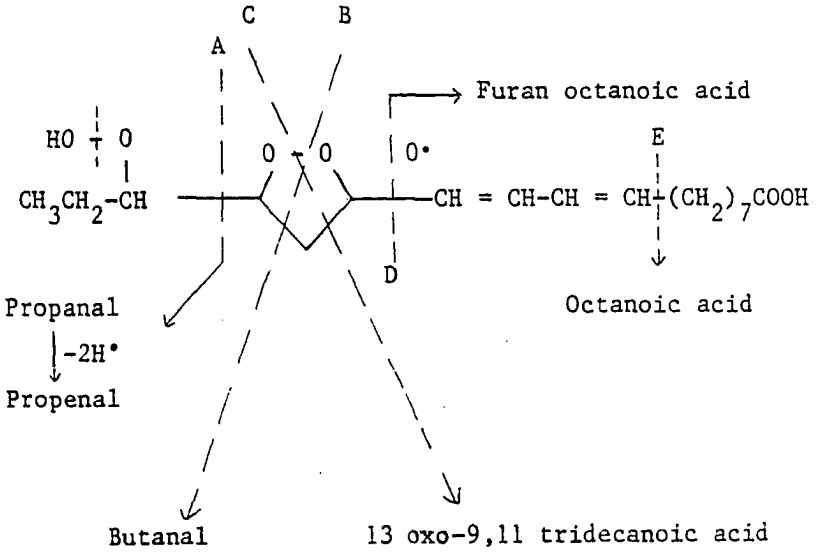
Recent studies have revealed the formation of dihydroperoxides, five-membered hydroperoxy epidioxides, hydroperoxy bicycloendoperoxides, and six-membered hydroperoxy epidioxides from the autoxidation or photosensitized oxidation of linoleic and linolenic acids (33,34,40,75,76,87). These secondary oxidation products are the results of further reactions of unsaturated hydroperoxides with oxygen. The volatile decomposition products of some of these oxidation products have been investigated (76,33, 40,34,79) and found to be, in general, similar to those from corresponding monohydroperoxides. In the case of hydroperoxy cyclic peroxides, they undergo thermal cleavage mainly between the peroxide ring and the hydroperoxide bearing the carbon (33). The following scheme shows the pathways of formation and decomposition of a hydroperoxy cyclic peroxide:



I: 13 linolenic acid hydroperoxide. $R_1 = (CH_2)_7COOH$.



(II)



II: 16-hydroperoxy-13,15-epidioxy-cis-9,trans-11-
-octadecadienoic acid

MATERIALS AND METHODS

Materials

Linoleic acid, linolenic acid, their methyl esters (99% pure), and soybean lipoxygenase (lipoxydase type I) were obtained from Sigma Chemical Co. (St. Louis, MO).

Tricaprin

Tricaprin was synthesized by reaction of methyl caproate (3M) and triacetin (1M) in the presence of 5% sodium methoxide. It was purified through alumina (57).

Dodecane

Dodecane (99% pure) was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI) and used directly.

Hydrogen peroxide

A 75% aqueous hydrogen peroxide was obtained by concentration of 30% solution under reduced pressure in a rotary evaporator. The temperature was kept under 50°C.

Florisil

Florisil (60-100 mesh, Floridin Co.) was purchased from Fisher Scientific Company. For purifying hexane, Florisil was activated at 300°C overnight. For column chromatography, Florisil was activated at 250°C overnight and 10% of its weight of water was added and allowed to equilibrate for 24 hours before use.

Hexane

Hexane (certified grade, Fisher Scientific Co.) was distilled slowly through a 100-cm column of porcelain saddles to remove high-boiling impurities, then passed through a column of activated Florisil (30 ml/g) to remove carbonyl impurities.

Ether

Ether was reacted overnight with $LiAlH_4$ and distilled fresh when needed.

2,4,6-Trichlorophenylhydrazine (TCPH)

TCPH was purified by recrystallization from ethanol as described by White and Hammond (97).

Ethanol

Ethanol was refluxed over aluminum foil and trace of iodine and distilled.

Tetrachloroethane (TCE)

TCE was purified by heating with lauroyl peroxide at 100° for 1 hr, distilled at reduced pressure, and a second time distilled under reduced pressure from 1-5-diphenylcarbohydrazide (66).

Alumina

Alumina (80-200) was purchased from Fisher Scientific Company and used directly.

Silica gel (G and GF)

Silica gel was purchased from Sigma and used directly.

Standards

A series of 2-ketones (C4 to C11), aldehydes (C5 to C10), 2-enals (C5 to C10), and 2,4-dienals (C6 to C10) were checked for purity by gas chromatography and distilled if necessary.

1-Bromo-2 cyclohexene

1-Bromo-2 cyclohexene was prepared by reaction of cyclohexene and N-bromosuccinimide (NBS) as reported by Ziegler et al. (98): 18.3 g of NBS in 75 cc of carbon tetrachloride (CCl_4) and 51.5 cc of cyclohexene was refluxed for about 1 hour, after which the white succinimide formed was filtered off. CCl_4 and the excess of cyclohexene were distilled at atmospheric pressure, and the residual cyclohexene bromide distilled under reduced pressure.

Cyclohexene hydroperoxide

Cyclohexene hydroperoxide was prepared according to Gunstone et al. (44): A mixture of bromocyclohex-2-ene (1.6 g, 10 mmole), cyclohexene (1.6 g, 19 mmole); 50 ml acetonitrile and hydrogen peroxide (75% aqueous solution, 1.6 ml, 50 mmoles) was kept at room temperature for 2 hours. The solution was then concentrated to 5 ml in a rotary evaporator and added to a silica gel column. The hydroperoxide was eluted with a mixture of 20% ethyl ether in petroleum ether and further purified by thin layer chromatography on a 0.5-mm thick plate. After development with the solvent system n-hexane/ether (70:30 v/v), the hydroperoxide band was

located by spraying the edges of the plates with a spray containing ammonium thiocyanate and ferrous sulfate (44). The band was then scraped off, eluted with ether and stored under nitrogen at -20°C.

Cyclopentene carboxaldehyde

Cyclopentene carboxaldehyde was prepared by periodate-osmium tetroxide oxidation of cyclohexene according to a procedure described by Pappo et al. (77): A mixture of 15 ml of ether, 15 ml of water, 0.405 g of cyclohexene, and 65.4 mg of osmium tetroxide (OsO_4) was stirred at 24-26° during the addition of 2.32 g of finely ground sodium periodate over a period of 40 min. Stirring at 24-26° was continued for 80 min, by which time the color had changed to yellow and considerable sodium iodate had separated. The mixture was extracted thoroughly with ethyl acetate, and the combined organic layers were filtered through sodium sulfate and concentrated to 2 ml in a rotary evaporator. The carbonyl was isolated by preparative thin layer chromatography (ether/hexane: 45:55 v/v)

13-Linoleic acid and 13-linolenic acid hydroperoxides

A linoleic acid hydroperoxide mixture enriched in the 13 isomer was produced using the soybean lipoxygenase procedure of Gardner (38). The reaction mixture (480 ml total volume) was 5.4 mM linoleic acid, 0.1% Tween 80, 5.0 mM borate, and 0.04 mg lipoxygenase/ml. Before lipoxygenase was added, the reaction solution was adjusted to pH 10 with KOH. Good oxygenation was ensured by using a sintered glass tube through which pure oxygen was bubbled into the reaction mixture. The oxidation proceeded at 21°C for 40 min at which time the reaction was adjusted to pH 4 with HCl

and immediately extracted with chloroform-ethanol 2:1. The chloroform layer was washed several times with water, dried over sodium sulfate, evaporated to a volume of 5 ml, and the hydroperoxide purified by thin layer chromatography on a 0.5-mm plate of silica gel GF. After being developed twice with the solvent system n-hexane/ether/acetic acid (60:40:1 V/V), the hydroperoxide band was located by UV, scraped off and eluted with methanol. The hydroperoxide band also was identified by spraying the edge of the plates with a spray containing ammonium thiocyanate and ferrous sulfate, as described by Gunstone et al. (44). The hydroperoxide solution was stored at -20°C under a nitrogen blanket until use. The same procedure was applied to linolenic acid to obtain a hydroperoxide mixture enriched in the 13-isomer.

9-Linoleic and 9-linolenic acid hydroperoxides

The method of Galliard and Phillips (37) as adapted by Chan et al. (14) was used to prepare the 9-hydroperoxide isomers of linoleic and linolenic acids. Briefly, the ammonium salt of linoleic or linolenic acid was made by adding ammonium hydroxide (2M, 40 ml) followed by Tween 80 to the fatty acid (750 mg) in a 1-l round bottom flask. Excess ammonia was displaced under a nitrogen stream until the pH of the solution was 9-9.5. Potato extract was prepared, combined with 0.5M acetate buffer (100 ml, pH 5.5) and added to the ammonium salt fatty acid solution. The solution was adjusted to 25°C and oxygen was bubbled through it for 60 sec at 5-min intervals. After 30 min, the reaction was stopped by acidification to pH 3 using 2M HCl, and the emulsion was extracted with ether. The ether extract was washed several times with water, dried over anhydrous $MgSO_4$,

and evaporated to 10 ml. The purification and storage conditions were the same as outlined previously for the 13-hydroperoxide isomers.

Hydroperoxides from the autoxidation of methyl linoleate and methyl linolenate

Autoxidation was done with 1 g of pure fatty acid methyl ester in a 40-ml beaker at 37°C in the dark. When the peroxide value was approximately 1000-1500, the autoxidized samples were dissolved in 5 ml of hexane, and the methyl ester hydroperoxides were isolated by chromatography on 0.5-mm plate of silica gel GF. After being developed twice with the solvent system hexane/diethyl ether (70:30 v/v), the hydroperoxides were located as before, scraped off, eluted with methanol, and stored at -20°C.

Methods

Preparation of the hydroperoxide solutions

The hydroperoxides previously prepared were freed of methanol or ether under nitrogen and dissolved in either tricaprin (cyclohexene hydroperoxide) or dodecane (fatty acid hydroperoxides).

Cyclohexene hydroperoxide

Fifteen grams of tricaprin were added to approximately 70 mg of cyclohexene hydroperoxide and mixed thoroughly. The tricaprin was kept liquid by immersion in a water bath set at 31-33°C. The solution was then distributed (ca. 0.5 g) over 28 constricted glass tubes (ϕ 10 mm, 80 mm). The tubes were made from 10-cm pieces of ϕ 10 mm glass tubing that had been soaked overnight in alcoholic KOH. The tubes were then flushed with

nitrogen and sealed under vacuum. Incubation temperatures were 55°C (for 24, 48, 72 hours, 1 week), 80°C and 130°C (for 24, 48, and 72 hours) in the dark, and 55°C in the presence of light (24, 48, 72 hours). Two tubes were not incubated and served as the control (To). At the end of each incubation term, 2 tubes were opened and analyzed. Similar preparations were made to investigate the effects of free fatty acids (caprylic acid), monoglycerides (monoolein) and metal ions (copper acetate). These factors were added (1%) to the tubes which were then sealed and incubated at 55°C.

Free fatty acids and methyl ester hydroperoxides

Each hydroperoxide was mixed thoroughly with dodecane in glass stoppered centrifuge tubes. The free fatty acid hydroperoxides formed an emulsion which was shaken for 2 min, centrifuged sufficiently to obtain a clear dodecane layer and transferred to another test tube leaving the excess of hydroperoxides in the bottom of the first test tube.

For linoleic acid 13-hydroperoxide, two portions of 90 mg in two 15-ml centrifuge tubes with glass stoppers were mixed with 10 ml of dodecane. The emulsions were centrifuged and the top 9 ml from each tube were collected, combined and mixed thoroughly. The concentration of the hydroperoxide was then determined by the peroxide value of the solution. The dodecane and hydroperoxide mixture was then distributed among 35 constricted glass tubes (each receiving ca. 0.4 ml): eight tubes to investigate the thermal effects, 21 tubes for metal ions and antioxidants effects, and three tubes with ethanol as additive. For each trial, one tube was saved as To (not decomposed) and duplicates were analyzed. Three tubes with no additives were saved as To. The additives were previously

dissolved in either ethanol (PG, TBHQ, copper acetate, iron II acetate) or dodecane (BHA, BHT, α-tocopherol) and added to the hydroperoxides in a molar ratio equal to 1. The tubes were then flushed with nitrogen and sealed at the point of constriction by a hand torch under vacuum.

The other hydroperoxides (9-18:2-OOH, 9-18:3-OOH, 13-18:3-OOH, Me 18:2-OOH, Me 18:3-OOH) solutions were prepared following basically the same procedure as for 13-18:2-OOH, but only the thermal effects were investigated. The concentrations of the hydroperoxides and the incubation conditions for each hydroperoxide are specified in the section Results and Discussion.

Thermal effects were investigated at 160°C (oven, in the dark for 6 hr), 80°C (oil bath, in the dark for 96 hr), 55°C (oven, in the dark for 6 days), and 40°C (oven, dark, 6 days). The samples containing the additives were decomposed at 55°C for 6 days.

Analysis of the decomposition products

After incubation, the tubes were opened and the samples analyzed. If they had to be stored after the thermal treatment and before analysis, this was done at -20°C for a period not exceeding 1 week.

For each sample, the extent of decomposition was determined by peroxide value, and the carbonyls were quantified using the method of White and Hammond (97):

To form the TCPHs from the carbonyls, the sample (0.1 to 0.2 g) was accurately weighed and placed in a 50-ml round bottom flask with 11 g octanone (internal standard), 0.1 g TCPH, and 0.5 g Florisil in 15 ml ether. Florisil catalyzed the reaction, allowing immediate derivatization

of the carbonyls with no added acid. The ether was evaporated in a rotary evaporator at a temperature below 25°C to avoid thermal breakdown of hydroperoxides. To separate the TCPH derivatives from dodecane and excess reagent, the dry Florisil-TCPH-dodecane mixture was slurried with hexane and packed on top of an 11-mm id x 33-cm column containing 9.5 g Florisil. The column was washed with 30 ml hexane/ether (99:1) which was discarded, and the TCPHs were collected in the next 45 ml. The solvent containing the derivatives was concentrated to ∿3 ml by rotary evaporation and transferred to a 15-ml centrifuge tube and evaporated to 60 µl under nitrogen in a 30°C water bath. A 1-µl sample was injected into the GC. For quantification, sample peak heights were compared with the internal standard (2-octanone). For identification, retention times were compared with those of known compounds. A chart mapping the retention times of the TCPHs of selected 2-ketones (C3-C10), aldehydes (C5-C10), 2-enals (C5-C10), and 2,4-dienals (C6-C10) using the same procedure outlined above, was prepared for this purpose.

Gas chromatography (GC)

For cyclohexene hydroperoxide investigations, the TCPHs were analyzed on a Varian Aerograph Series 1520 gas chromatograph equipped with a hydrogen flame detector. A fused silica capillary column (30 m) coated with SE 30 (Supelco, Belfonte, PA) was used. The column temperature was programmed from 50° to 250°C at 10°C/min.

For fatty acid hydroperoxide investigations, the TCPHs were analyzed on a Varian Model 3700 gas chromatograph equipped with a hydrogen flame detector. A fused silica capillary column (SPB-1 15 m) was purchased from

Supelco. The column temperature was programmed from 50° to 250°C at 12°C/min.

Peroxide value

Peroxide values of the samples before and after decomposition were determined by the method of Hamm et al. (48).

RESULTS AND DISCUSSION

Thermal Decomposition of Cyclohexene Hydroperoxide

Thermal decomposition products of cyclohexene hydroperoxide were previously investigated in this laboratory using Schwartz et al's. procedure (87) to isolate the carbonyls formed. Beside cyclohexenone, the major carbonyl expected and found, some other minor carbonyls resulting from the β-scission of the hydroperoxide (such as cyclopentene carboxaldehyde) were also observed. However, these compounds might have been the result of an acid-catalyzed scission of the hydroperoxide rather than being thermal decomposition products. Phosphoric acid present in the reaction column would decompose the hydroperoxide resulting in the formation of the same kind of carbonyls expected from the thermal β-scission of the hydroperoxide.

Recently, in this laboratory, White and Hammond (97) developed a rapid and sensitive method for the quantification of carbonyls in oxidized fats as trichlorophenylhydrazones. No acid was necessary for the formation of the TCPH derivatives because it was observed that Florisil catalyzed the reaction. Because of the sensitivity of the method and the absence of acid as catalyst, it was thought of interest to use it for the identification and quantification of the carbonyls resulting from the thermal decomposition of cyclohexene hydroperoxide. This approach would provide some information about the decomposition of allylic hydroperoxides and the factors that control the extent of the β-scission.

Cyclohexene hydroperoxide in solution in tricaprin (3 mg/g) was decomposed anaerobically under different conditions. Temperatures of

55°C, 80°C, 130°C at times varying from 12 hours to 1 week were investigated. Factors such as free fatty acids (1% caprylic acid), monoglyceride (monoolein 1%), and metal ions (copper acetate) were added to the tricaprin-cyclohexene hydroperoxide mixture to investigate their effects.

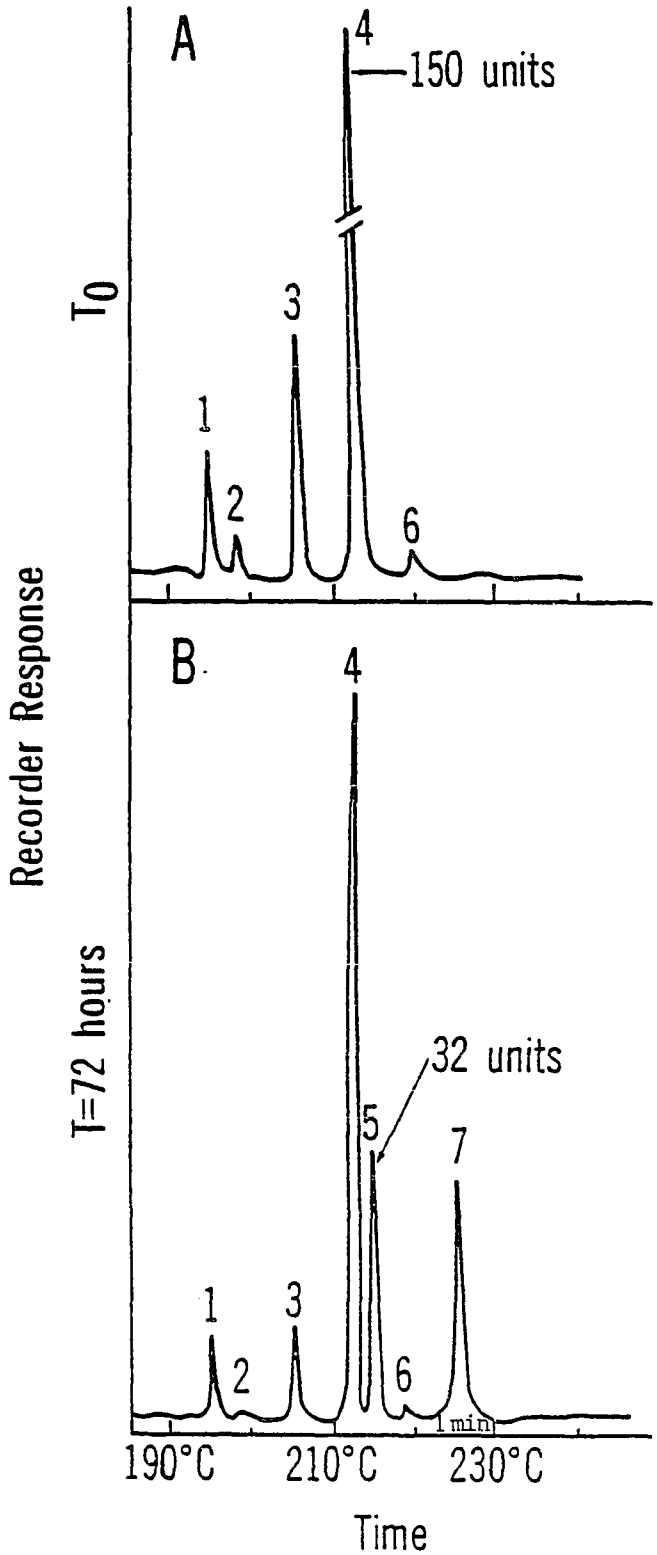
The results obtained showed that invariably, the only carbonyl detectable from decomposition of the hydroperoxide was 2-cyclohexenone. A typical chromatogram obtained from cyclohexene hydroperoxide decomposition is shown in Figure 1. The retention time of cyclopentene carboxaldehyde was determined using the same column procedure and found to be slightly shorter than the retention time of cyclohexenone. When injected together, the 2 carbonyls presented two clearly separated peaks. Obviously, cyclopentene carboxaldehyde could have been detected by the procedure if it had been present as a decomposition product of the hydroperoxide.

Attempts to quantify the cyclohexenone produced resulted in amounts much smaller than expected. To check the sensitivity of the method for cyclohexenone, the pure compound was tested in the presence of the same amount of an internal standard (heptanone). A very poor GC response was observed for cyclohexenone. Even when the reaction was carried out in a test tube for a prolonged time of 1 or 2 hours, and the column separation step was omitted, the same low results for cyclohexenone were obtained. It was thought that the TCPH derivative of cyclohexenone might decompose under our GC conditions. However, lowering the temperatures of the injector and detector from 250°C to 200° and even lower, did not bring

Figure 1. Chromatograms of carbonyl-TCPHs from cyclohexene hydroperoxide decomposed at 80°C for 72 hours

A: Cyclohexene hydroperoxide before decomposition (To)
B: Cyclohexene hydroperoxide decomposed at 80°C for 72 hrs

1,2,3,4,6: Carbonyl impurities from solvents and tricaprin
5: Internal standard (2-heptanone)
7: 2-Cyclohexenone


A
4
150 units
3
1
2
6
T0
B
4
32 units
5
7
1
3
2
6
1 min
T=72 hours
Recorder Response
190°C
210°C
230°C
Time

about any improvement. Reducing the length of the column from 30 m to 20 m decreased the retention time of all the carbonyls but did not affect the GC response. One explanation for this behavior of cyclohexenone would be a poor reactivity with the TCPH due to a steric hindrance effect. Nevertheless, by reacting various amounts of cyclohexenone and heptanone with TCPH, a linear GC response was observed for both ketones, and it was possible to calculate a correction factor for cyclohexenone based on the GC response for heptanone. This factor was equal to 0.11 and represents the ratio of the height of the peak obtained for cyclohexenone over the height of the peak of the same amount of heptanone. The molar yield of cyclohexenone produced from cyclohexene hydroperoxide decomposed at 80° for 72 hours was ∿60%.

The method is sensitive to about 10^{-7} g of carbonyl, and usually enough decomposed hydroperoxide was analyzed so that a carbonyl formed with a yield of 1% or more would be possibly detected. One can conclude from these observations that cyclohexene hydroperoxide decomposed to give only 2-cyclohexenone with less than 1% β-scission carbonyls under the conditions investigated.

Quantification of Carbonyls Produced From the Decomposition of Fatty Acid Hydroperoxides

The method of White and Hammond (97) for the quantification of carbonyls in oxidized soybean oil as trichlorophenylhydrazones was extended to the analysis of carbonyls formed by decomposition of linoleic and linolenic acid hydroperoxides. The first step in White's procedure

was omitted in this investigation, since the purpose of the first column was to remove interfering hydrocarbons from the fat sample.

The decomposition of linoleic and linolenic acid hydroperoxides and their methyl esters was investigated in dodecane as solvent instead of tricaprin used for cyclohexene hydroperoxide. Impurities observed in tricaprin interfered with the analysis of carbonyls formed from the hydroperoxides and it was not possible to remove these impurities even after repeated purification of tricaprin through alumina.

Preliminary trials showed that incubating the hydroperoxides at 55°C for 6 days, 80°C for 96 hours, and 160°C for 6 hours was enough to lead to a fairly complete decomposition of the hydroperoxides. It was also observed from these trials that metal ions and antioxidants did have some effects on the formation of carbonyls. Therefore, the effects of these factors were investigated in incubations at 55°C for 6 days.

Corrections and Response Factors Used in the Calculations of the Yields

When the carbonyl standards (2-ketones, saturated aldehydes, 2-enals and 2,4-dienals) were applied to the column, it was observed that the TCPHs of the saturated aldehydes (only these carbonyls) formed double peaks under our GC analysis conditions. The secondary peak was usually smaller (20%) than the main peak. Previous workers also reported the formation of double peaks in the gas-liquid chromatography analysis of 2,4-dinitrophenylhydrazones (59,67) or 2,4,6-trichlorophenylhydrazones (97) of carbonyl compounds. Kallio et al. (59) reported that the occurrence of the double peaks (possibly *syn*- and *anti*-isomers) depends on the

compound, but especially on the solvent employed: clear double peaks were formed when hexane was the solvent, which is the case in this study. Johnson and Hammond (58) also reported double-peak formation, but when all metal was removed from the GC column and replaced by glass, the double peaks disappeared. This observation suggested that possible rearrangement products, rather than syn-anti-isomerization, were responsible for the double peaks. White and Hammond (97) did not report the formation of such double peaks, possibly because they used cyclohexane as solvent and a different capillary column.

In these investigations, the main peak as well as the secondary peak were considered (added together) in the case of the saturated aldehydes. In the case of hexanal, the identity of the secondary peak was checked by GC-MS and found to be a saturated C6 carbonyl; in other words, a hexanal isomer.

During this preliminary work, it was also observed that the use of different amounts of carbonyl standards (to check the accuracy of the procedure) showed a fairly linear GC response with the amount of carbonyl introduced; however, the GC response decreased significantly with the increase of the carbon chain length of the carbonyls. These carbonyls were, in general, eluted at higher temperatures. Correction factors were then calculated for carbonyls eluting after 2-octanone (the internal standard). They were determined by taking the ratio of the GC response for the carbonyl over the GC response for the internal standard when the same amounts were introduced.

The third and last correction adapted in the calculation of the amounts of the carbonyls produced accounts for artifact production from decomposition of hydroperoxides through the Florisil column. White and Hammond (97) reported that some hydroperoxide scission had occurred in their TCPH procedure. In the present investigations, it was observed that when linoleic acid hydroperoxide (in dodecane) was passed through the column, considerable amounts of scission product (hexanal) were obtained. The possibility of hexanal formation from the hydroperoxide during its preparation and prior to passage through the column was ruled out since the TLC purification of the hydroperoxide eliminated any hexanal that would be present. Indeed, the Rf values for the hydroperoxide and the carbonyl where checked under our TLC conditions and found clearly distinct. These artifacts might have formed not only by passage through the column, but also prior to the column step, during the preparation of the TCPH derivatives.

Because not all the samples were totally decomposed prior to the column analysis, artifact production from the remaining hydroperoxide should be considered. To account for this, the amount of carbonyls determined for partially decomposed samples was corrected by subtracting the amount of artifacts that the remaining hydroperoxides would produce. For this purpose, a sample that had not been incubated (To) was passed through the column, and the amounts of carbonyls produced from the hydroperoxide were determined.

Thermal Effects

Decomposition of linoleic acid 9- and 13-hydroperoxides at 40°C, 55°C, 80°C, and 160°C resulted, as shown in Tables 3 and 4, in hexanal and 2,4-decadienal as the only two quantifiable aldehydes identified. Figure 2 shows a typical chromatogram of the carbonyl-TCPHs obtained from the decomposition of linoleic acid hydroperoxides. Peak numbers 1, 1a, 2, and 7 correspond to hexanal, 2-heptanone (the internal standard), and 2,4-decadienal, respectively. The two peaks eluting before hexanal were carbonyl impurities coming from the solvents (hexane and ether), the peak shown after 2-heptanone is also an impurity resulting from TCPH.

2,4-Decadienal was produced essentially at high temperatures (80°, 160°C). A statistical analysis using the Duncan multiple range test shows that the yields of hexanal at 40°C, 55°C, and 80°C were significantly higher than those obtained at 160°C for both hydroperoxides.

The yield of these carbonyls (5.5% to 11.7% for hexanal, 0% to 6% for 2,4-decadienal) were much lower than those reported by Chan et al. (14) who decomposed neat linoleate 9- and 13-hydroperoxides at 160°C in the injection port of a gas chromatograph and obtained yields of 67% to 80%. They suggested their yields were high because they used anaerobic conditions in the absence of unreacted linoleate. However, since our peroxides were decomposed under similar conditions, it is not clear how to account for the difference in yields. These results are much closer to those reported by other investigators who decomposed hydroperoxides under various conditions and obtained yields between 10% and 20% (36,64). Grosh (41) reported a yield of 6% for hexanal when a mixture of linoleic acid

Table 3. Yields (Mol. %) of carbonyls produced from linoleic acid 13-hydroperoxide[a] decomposed at various temperatures

Temp. of incubation	Time of incubation	% Decomposition	Hexanal (Mol %)[c]	2,4-Decadienal[b] (Mol %)[c]
40°C	6 days	50%[d]	10.4 A	0.0 A
55°C	6 days	100%	11.7 A	1.3 A
80°C	96 hours	100%	8.5 A	4.3 B
160°C	6 hours	100%	7.1 B	4.1 B

[a]Concentration of the hydroperoxide in dodecane was 4.2 mg/g.

[b]Correction factor = .25.

[c]Means that share the same letter are not significantly different ($p<0.05$).

[d]Corrected for artifacts produced.

Table 4. Yields (Mol. %) of carbonyls produced from linoleic acid 9-hydroperoxide[a] decomposed at various temperatures

Temp. of incubation	Time of incubation	% Decomposition	Hexanal (Mol %)[c]	2,4-Decadienal[b] (Mol %)[c]
40°C	6 days	40%[d]	11.7 A	0.0 A
55°C	6 days	80%[d]	10.5 A	0.0 A
80°C	96 hours	100%	7.2 A	5.6 B
160°C	6 hours	100%	5.5 B	5.7 B

[a]The concentration of the hydroperoxide in dodecane was 3.1 mg/g.

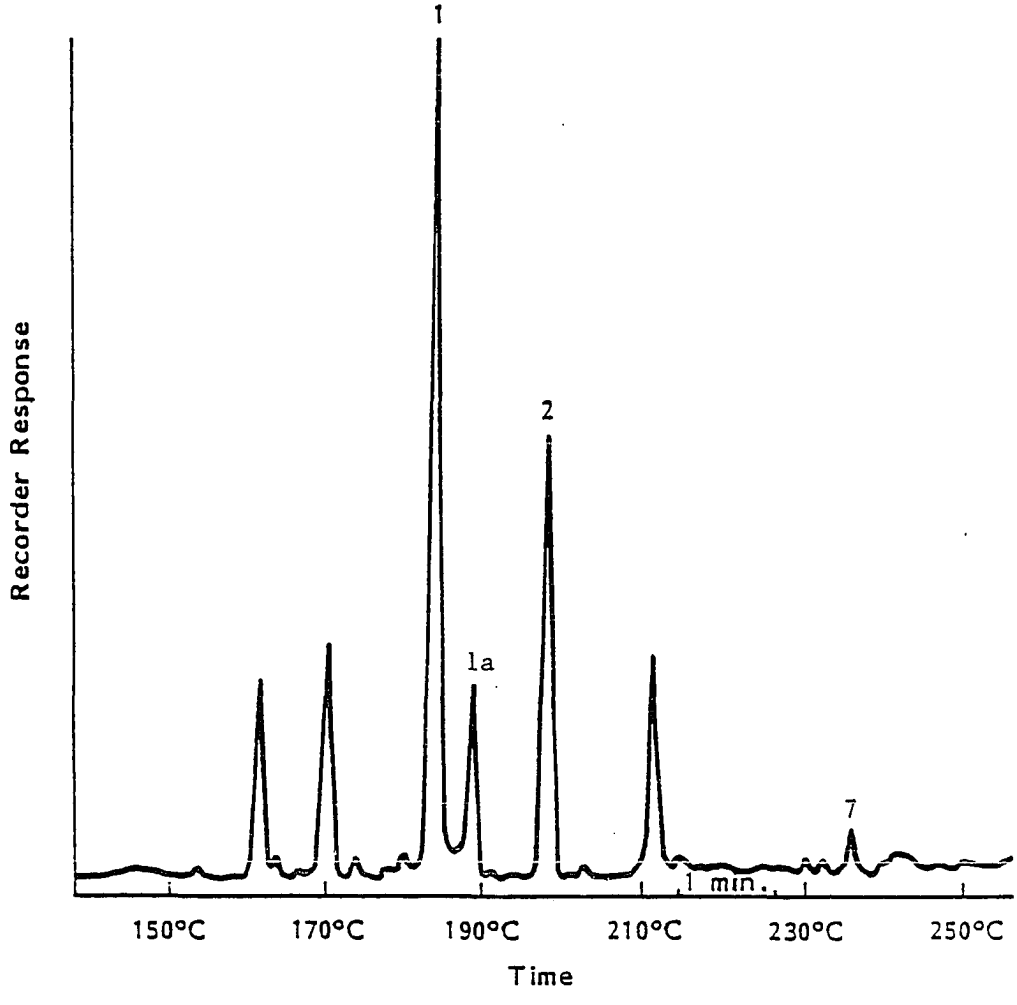
[b]Correction factor = .25.

[c]Means that share the same letter are not significantly different ($p<0.05$).

[d]Corrected for artifacts produced.

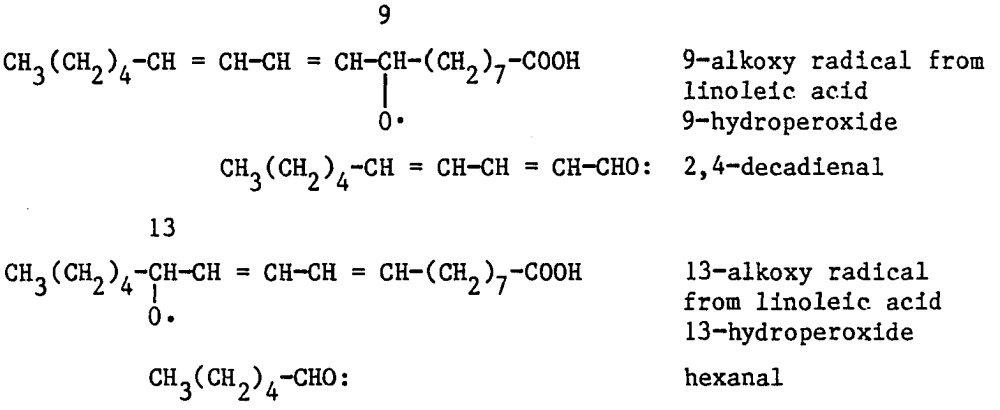
Figure 2. Chromatogram of carbonyl-TCPHs from the decomposition of linoleic acid 13-hydroperoxide at 55°C for 6 days. GC capillary column is 15 m SPB1, attenuator 64, electrometer 10^{-11}

1,1a: Hexanal
2: 2-Octanone (internal standard)
7: 2,4-Decadienal


1
2
1a
7
Recorder Response
1 min.
150°C
170°C
190°C
210°C
230°C
250°C
Time

hydroperoxide isomers (75% 13-OOH, 25% 9-OOH) was decomposed at ambient temperature in the presence of ascorbic acid.

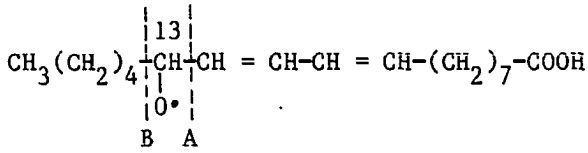
The carbonyls obtained are those expected from a simple carbon-carbon bond scission adjacent to an alkoxy radical at both positions 9 and 13:



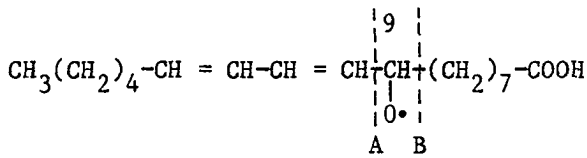
According to the scheme outlined above, 2,4-decadienal is expected to form exclusively from the 9-hydroperoxide and hexanal from the 13-isomer. However, our results showed (especially at high temperatures) that both isomers formed hexanal and 2,4-decadienal, which means that a positional isomer of linoleate hydroperoxide can give rise to products normally attributed to the other isomer. These apparently anomalous results were also observed by Chan et al. (14) when they decomposed pure individual isomers of linoleate hydroperoxides at 160°C and obtained hexanal and 2,4-decadienal from both isomers. According to these authors, this phenomenon can be explained by the rapid isomerization of the individual isomers prior to decomposition. Chan et al. (13,15) have already shown that both the 9- and 13-isomers of linoleate hydroperoxide undergo isomerization readily at low temperatures. Thus, sufficient isomerization

could have taken place during the preparation, purification and incubation of the hydroperoxides to account for the results observed in this study.

The almost exclusive formation of hexanal at lower temperatures and the relatively high yields of 2,4-decadienal at higher temperatures was also reported by previous researchers (64,93). Swoboda and Lea (93) explained this effect of temperature by further selective oxidation of 2,4-decadienal. This reasoning cannot hold in our study, for the hydroperoxides were decomposed essentially under anaerobic conditions. The most plausible explanation would be the one proposed by Kimoto and Gaddis (64) which is based on a selective course of scission of the hydroperoxides. The carbon-carbon bond between an alkoxy radical and a double bond was considered to be most easily cleaved (side A). Thus, the 13-hydroperoxide yields predominant amounts of hexanal and the 9-hydroperoxide can form a little 2,4-decadienal spontaneously, but mostly the semi-aldehyde.



$CH_3(CH_2)_4-CHO$: hexanal



$OHC-(CH_2)_7-COOH$

At higher temperatures, scission takes place also at the other side (B) of the alkoxy radical and the formation of 2,4-decadienal becomes significant.

No 2-alkenals were observed, but considerable amounts of carbonyls with retention times not matching those of any of the normal aldehydes, 2-enals, 2,4-dienals or 2-ketones standards, were formed exclusively at 160°C. A typical chromatogram of these unknown carbonyls showing their elution temperature is presented in Figure 3. Table 5 shows the molar yields of these carbonyls.

Metal Ions Effects

Linoleic acid 13-hydroperoxide was decomposed at 55°C for 6 days in the presence of copper acetate and iron acetate which were previously dissolved in ethanol and added to the hydroperoxide solution (4.2 mg/g of dodecane) to give a molar ratio concentration of 1. The results were compared with a sample decomposed under the same circumstances in the presence of ethanol only. Table 6 shows that metal ions gave higher yields of both hexanal and 2,4-decadienal. The same pattern of unknown carbonyls reported previously at 160°C was also observed under these conditions. These carbonyls were formed in much higher yields with Cu^{++} than with Fe^{++} (Table 6). Metal ions and especially cupric ions (Cu^{++}) are well-known as powerful decomposers of hydroperoxides (64,65,68). These results are in agreement with previous work of Kimoto and Gaddis (64) who showed a similar effect of heat and cupric stearate when they decomposed autoxidized trilinolein in sealed tubes. However, we were not

Figure 3. Chromatogram of the unknown carbonyls observed during decomposition of linoleic acid hydroperoxides at 160°C. GC capillary column is 15 m SPB-1, attenuator 64, electrometer setting 10^{-11}

1,1a: Hexanal
2: 2-Octanone (internal standard)
3,4,5,6: Unknown carbonyls
7: 2,4-Decadienal

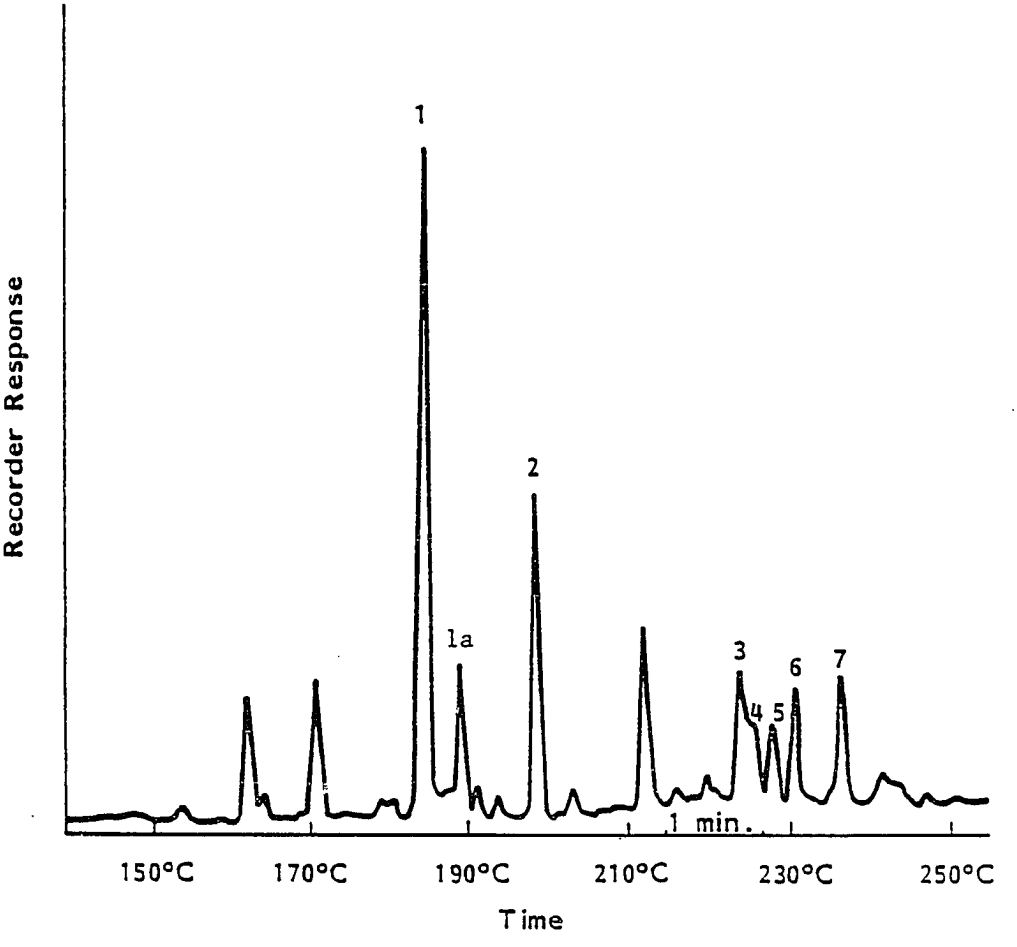

Recorder Response
1
1a
2
3
4
5
6
7
1 min.
150°C
170°C
190°C
210°C
230°C
250°C
Time

Table 5. Yields (Mol. %) of unidentified carbonyls[a] produced from linoleic acid hydroperoxides decomposed at 160°C for 6 hours

	Peak No. 3	4	5	6	
	Elution temperature 223°C	225°C	227°C	230°C	Total
13-hydroperoxide	2.0	1.5	1.3	2.0	6.8
9-hydroperoxide	2.6	1.7	1.7	3.5	9.5

[a]Since their retention times were close to 2,4-nonadienal, their yields were calculated assuming that they have the MW of 2,4-nonadienal (138). Correction factor = .45.

Table 6. Yields (Mol. %) of carbonyls produced from linoleic acid 13-hydroperoxide[a] decomposed in the presence of metal ions

Trial	Hexanal[c]	2,4-Decadienal[c,d]	Unknown carbonyls[b] 223°C	225°C	227°C	230°C	Total
None[e]	6.3 B	0.9 A	-	-	-	-	1.5
Cu^{++} Acet.	9.1 A	4.7 B	8.9	6.3	7.6	10.6	33.4
Fe^{++} Acet.	7.6 B	2.0 C	3.0	1.9	2.7	5.6	13.2

[a]The concentration of the hydroperoxide in dodecane was 3.1 mg/g.

[b]Yields calculated assuming that MW = 138, correction factor = .45 (values for 2,4-nonadienal).

[c]Means that share the same letter are not significantly different ($p<0.05$).

[d]Correction factor = .25.

[e]Only ethanol was added (5%).

also to identify any 2-alkenals, even though the authors (64) obtained 2-heptenal in amounts similar to those of hexanal.

Antioxidants Effects

Several well-known antioxidants were investigated under the same conditions used for metal ions. Butylated hydroxytoluene (BHT), propyl gallate (PG), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and α-tocopherol (α-Toco.) were dissolved in either ethanol (PG, TBHQ) or dodecane (BHA, α-Toco., BHT) and added to the hydroperoxides in a molar ratio equal to 1. The results are presented in Table 7. Because the antioxidants were dissolved in either ethanol or dodecane, their effects were compared with control samples: dodecane only or dodecane in the presence of 5% ethanol. In the presence of ethanol, the hydroperoxides decomposed to a lesser extent (30%). Possibly a hydrogen binding between the alcohol and the hydroperoxide would form a complex that makes the hydroperoxide more stable. In general, the antioxidants showed a tendency to accelerate the decomposition of hydroperoxides as has been previously reported by Privett (81) and by Hill et al. (54). This was more evident in preliminary investigations where the hydroperoxides were decomposed for shorter times (3, 5 days). It was noted that α-tocopherol and TBHQ were the most effective in accelerating hydroperoxide decomposition. The results show that hexanal and 2,4-decadienal were the only carbonyls identified. Some antioxidants (BHA, α-Toco.) gave lower yields of hexanal, and PG and TBHQ resulted in higher yields of 2,4-decadienal. The results obtained with antioxidants dissolved in ethanol should be considered cautiously because of the possible

Table 7. Yields (Mol. %) of carbonyls produced from linoleic acid 13-hydroperoxide[a] decomposed in the presence of various antioxidants

Trial	% Decomposition[b]	Hexanal[c]	2,4-Decadienal[c,d]
None (dodecane)	100%	11.7 A	1.3 A
BHA (in do.)	73%	7.9 B	0.4 A
BHT (in do.)	63-67%	9.9 A	0.4 A
α-Toco. (in do.)	100%	5.6 B	0.3 A
None (ethanol)[e]	30-35%	6.3 B	0.9 A
PG (in EtOH.)	73%	5.9 B	3.2 B
TBHQ (in EtOH.)	100%	4.5 B	2.5 B

[a]The concentration of the hydroperoxide in dodecane was 3.1 mg/g.

[b]Samples not completely decomposed were corrected for artifacts production.

[c]Means that share the same letter are not significantly different ($p<0.05$).

[d]Correction factor = .25.

[e]Only ethanol was added (5%).

interactions of the alcohol with the hydroperoxides or intermediate decomposition products. However, when only the antioxidants dissolved in dodecane were considered, it is clear that carbonyls were produced in lower yields. To account for this, one can speculate that the alkoxy radicals that are intermediate in the decomposition and scission of hydroperoxides were reduced to alcohols by a hydrogen donated by the antioxidant, thus inhibiting further scission of the alkoxy radicals to carbonyls. Another possibility would be a radical addition of the hydroperoxide to the antioxidant. Gardner et al. (39) have already shown that

either linoleic acid hydroperoxide or methyl linoleate hydroperoxide reacts anaerobically with either α-tocopherol or its model compound 2,2,5,7,8-pentamethyl-6-hydroxychroman to form principally an additional compound of the two reactants. The mechanism appears to be free radical addition of alkoxy radicals from the hydroperoxide and chromanoxy radicals from tocopherol. Even though more systematic investigations using various concentrations of antioxidants would lead to more conclusive results, it is clear that antioxidants do have some effect on the decomposition of hydroperoxides to carbonyls. Therefore, it is important in choosing an antioxidant to consider its effects on scission product yields, as well as its effect on the accumulation of hydroperoxides during autoxidation.

Carbonyls Produced From the Decomposition of Methyl Linoleate and Methyl Linolenate Hydroperoxides Produced by Autoxidation

Methyl linoleate and methyl linolenate hydroperoxides were decomposed at 160° and 55° for 6 hours and 6 days, respectively. Table 8 shows the molar yields of the carbonyls identified. At the lower temperatures, when compared with the free fatty acid hydroperoxides, the methyl esters were found to be much more stable (only 5% decomposition). Possibly the free carboxyl group acted as catalyst of hydroperoxide decomposition. Most of the aldehydes identified in this study were also reported by Frankel et al. (32) from the decomposition of pure hydroperoxides from autoxidized and photosensitized oxidized methyl linoleate and linolenate. At 160°C, methyl linoleate hydroperoxide formed hexanal and 2,4-decadienal as major products. Their yields (8% and 5%, respectively) were comparable with

Table 8. Carbonyl yields from the decomposition of methyl linoleate,[a] methyl linolenate[b] hydroperoxides and linolenic acid[c] 13-hydroperoxide

Aldehydes	Carbonyl yields (Mol. %)	
Me 18:2-OOH	160°C (100% decomposition)	55°C (5% decomposition)[d]
Pentanal	1.1	-
Hexanal	7.8	8.4
2-Heptenal	1.1	-
2,4-Decadienal	5.8	-
Me 18:3-OOH		
Propanal	4.0	3.6
Butanal	tr. (<1%)	tr. (<1%)
2-Butenal	1.0	-
2-Pentenal	1.3	-
2-Hexenal	tr. (<1%)	-
2,4-Heptadienal[e]	3.7	4.1
18:3-13-OOH[f]		
2-Pentenal	2.5	6.9
2/3-Hexenal	3.2	-

[a]Concentration of the hydroperoxide in dodecane = 4.4 mg/g.

[b]Concentration in dodecane = 5 mg/g.

[c]Concentration in dodecane = 2.3 mg/g.

[d]Corrected for artifacts produced.

[e]Correction factor = .45.

[f]Eighty percent decomposition at 55°C.

those reported previously for the free fatty acid hydroperoxides. Pentanal and 2-heptenal were produced as minor products. 2-Heptenal probably arises from the 12-hydroperoxide that has been identified among photooxidized linoleate hydroperoxides. Pentanal was also reported by

Frankel et al. (32); however, its presence at this relatively significant level was not expected. Seemingly, the hydroperoxides formed by autoxidation were a more complex mixture than the 9- and 13-isomers formed enzymatically. The formation of pentanal cannot be explained by the classical cleavage mechanism of linoleate hydroperoxides. It is possible that it resulted from the decomposition of secondary oxidation products of linoleate hydroperoxides or from a further oxidation of the final scission products. Indeed, secondary oxidation of aldehydes may occur by reaction with the same peroxy radical formed by carbon-oxygen fission supported by Chan et al. (14) to explain the isomerization between the 9- and 13-linoleate hydroperoxides.

A mechanism for oxidative degradation of aldehydes postulated without direct experimental evidence proceeds through a peracid intermediate and would explain the formation of lower aldehydes (41):

$$R - CH_2 - CHO \xrightarrow{O_2} R - CH_2 - CO_3H \longrightarrow R - CH_2COOH$$

$$\longrightarrow RCHO + HCOOH.$$

Pentanal would then originate from further oxidation of hexanal. However, when ^{14}C-labeled hexanal was oxidized in soybean oil, hexanoic acid was produced at 50°, but no lower aldehydes were produced (73).

The mixture of methyl linolenate hydroperoxides decomposed at 160° gave rise to a larger number of carbonyls than did methyl linoleate. Among them, propanal and 2,4-heptadienal were the major products. The origin of propanal would be the 16-hydroperoxide and 2,4-heptadienal would be a scission product of the 12-hydroperoxide isomer. Other carbonyls

formed as minor products were butanal (traces), 2-butenal (1.0%), 2-pentenal (1.3%), and 2-hexenal (traces). Autoxidation of linolenate leads to the formation of 32% 9-, 11% 12-, 11% 13-, and 46% 16-OOH (32). In this study, the relatively high yield found for propanal is in agreement with the high concentration of its precursor (the 16-OOH). However, for the other carbonyls, their yields do not match their respective precursor hydroperoxides. The products expected from the decomposition of the 9-hydroperoxide (2,4,7-decatrienal) and the 13 isomer (2- or 3-hexenal) are completely absent or present as traces. Further discussion of the decomposition of these 2-hydroperoxides is presented in the following section. 2-Butenal might be the decomposition product of the 15-hydroperoxide identified in photosensitized oxidized methyl linolenate.

Butanal and 2-pentenal might have been formed from the decomposition of secondary oxidation products of linolenate hydroperoxides. For example, butanal can form from the decomposition of methyl 16-hydroperoxy-13, 15-epidioxy-cis-9, trans-11-octadecadienoate as presented on page 28.

When compared with linoleate, linolenate hydroperoxides quantitatively formed less total carbonyl yield, indicating that the problem of the potency of the off-flavors produced in oils containing linolenic acid might be related to the nature of the carbonyls produced rather than to high yields. However, the carbonyls reported in this study are only those best amenable to our column and GC detection technique and their relative concentration is probably also affected by their thermal stability. The fact that the carbonyls expected from the 9-hydroperoxide decomposition

were not detected is a good example. The possibility that linolenic acid yields a greater amount of scission products or more stable scission products than linoleic acid has been suggested lately by Hammond (49) after he converted the carbonyl analysis of oxidized soybean oil by White and Hammond (97) into relative flavor potency by the data of Dixon and Hammond (18). The flavor potency of the products from linolenic acid was found to be higher than that from linoleic acid, but not by a large amount. Based on its percentage in the oil and its rate of oxidation, linolenic acid seems to give greater yield of carbonyls than expected.

In this study, if one supposes that the carbonyl yield from the 9-hydroperoxide is the same as that from the 16-isomer (4%) and that no polymerization of the carbonyls had occurred under the incubation conditions, linolenate hydroperoxides still do not seem to give higher yields of carbonyls than linoleate.

Carbonyls Produced From 9- and 13-Linolenic Acid Hydroperoxides

9- and 13-Linolenic acid hydroperoxides were produced enzymatically and decomposed at 160° for 6 hours and 55°C for 6 days. Table 8 shows that 2-pentenal and 2- or 3-hexenal were the only two quantifiable carbonyls that resulted from the decomposition of the 13-isomer at 160°C; however, only 2-pentenal was formed when the hydroperoxide was decomposed at 55°C. Hexenal is the expected product of decomposition of the 13-hydroperoxide, but the formation of 2-pentenal is not so easily explained.

The decomposition of the 9-hydroperoxide led to no significant yield of carbonyls. Peers et al. (79), however, identified 2,4,7-decatrienal as a major carbonyl from thermally decomposed pure 9-hydroperoxide. Even though there is no reason for our procedure not to detectg this tridecadienal, this possibility should be checked by using pure 2,4,7-decatrienal as a standard.

It does not seem that the isomerization found in the case of linoleic acid had occurred with linolenic acid, for it would result in hydroperoxide isomers (12- and 13-) whose decomposition products would be identifiable. Peers et al. (79) also found little evidence of isomerization between the various positional isomers of linolenic acid hydroperoxides during the process of their decomposition. Why isomerization occurs with oleate hydroperoxides and linoleate hydroperoxides but not with linolenate hydroperoxides remains unclear.

SUMMARY

Cyclohexene hydroperoxide produced by reaction of cyclohexene bromide with hydrogen peroxide was dissolved in tricaprin (3 mg/g) and decomposed under vacuum to investigate the effects of various decomposition conditions on the carbonyls formed by β-scission. The carbonyls were isolated as 2,4,6-trichlorophenylhydrazones and quantified by gas chromatography. The only carbonyl detectable was 2-cyclohexenone with less than 1% β-scission carbonyl products. The molar yield of cyclohexenone produced was ∿60%.

Linoleic acid and linolenic acid 13-hydroperoxides produced by soybean lipoxygenase, linoleic and linolenic acid 9-hydroperoxides produced by potato lipoxygenase, and methyl linoleate and methyl linolenate hydroperoxides produced by autoxidation were dissolved in dodecane and decomposed anaerobically at various temperatures to investigate the effects of temperature on the yields of carbonyls produced. Linoleic acid 13-hydroperoxide also was decomposed at 55°C in the presence of metal ions (copper acetate, iron II acetate) and antioxidants (BHA, BHT, PG, α-toco., TBHQ). At lower temperatures (40° and 50°C) only hexanal was identified from the decomposition of both linoleic acid 9- and 13-hydroperoxides. At higher temperatures (80°C, 160°C), both hydroperoxides gave rise to hexanal and 2,4-decadienal. The molar yields of these 2 carbonyls varied from 5% to 12% for hexanal and 0% to 6% for 2,4-decadienal. Sufficient isomerization of the 2 hydroperoxides could have taken place during their preparation and incubation to account for the production of hexanal and 2,4-decadienal from either hydroperoxide. A selective course of scission

of the hydroperoxides where the carbon-carbon bond between the alkoxy radical and a double bond is considered to be the most easily cleaved, would be the reason for the exclusive formation of hexanal at lower temperatures.

Linoleic acid 13-hydroperoxide decomposed in the presence of Cu^{++} and Fe^{++} gave higher yields of hexanal and 2,4-decadienal. The same unknown carbonyls observed at higher temperatures (160°C) were also formed in the presence of metal ions. These unknown carbonyls were formed in much higher yields with Cu^{++} (33.4%) than with Fe^{++} (1.51%).

The presence of antioxidants accelerated peroxide decomposition and resulted in lower yields of hexanal. α-Tocopherol and TBHQ produced the lowest yields (5.6% and 4.5%, respectively). PG and TBHQ which were dissolved in ethanol before they were added to the hydroperoxides resulted in the formation of slightly higher yields of 2,4-decadienal. The antioxidants dissolved in dodecane (BHA, BHT, α-Toco.) formed less carbonyls than did the control sample.

The methyl ester hydroperoxides were found to be much more stable than the free fatty acid hydroperoxides. Methyl linoleate hydroperoxides formed hexanal and 2,4-decadienal as major products in yields comparable to those obtained from the 9- and 13-hydroperoxides. Pentenal and 2-heptenal were present as minor products (1.1% at 160°C).

The major carbonyls formed from methyl linolenate hydroperoxides decomposed at 160° and 55°C were propanal (4%) and 2,4-heptadienal (3.7%) resulting from the decomposition of the 16- and 12-hydroperoxides. Other carbonyls formed at yields $\leq$1% were butanal, 2-butenal, 2-pentenal, and 2-

or 3-hexenal. Except for 2- or 3-hexenal, the presence of these minor carbonyls could not be accounted for by the classical scheme of scission of simple hydroperoxides.

Linolenic acid 13-hydroperoxide decomposed at 160° formed 2- or 3-hexenal (3.2%) and 2-pentenal (2.5%), but only 2-pentenal was produced at 55°C. No quantifiable carbonyls were obtained from the decomposition of linolenic acid 9-hydroperoxide.

GENERAL CONCLUSIONS

1. Thermal decomposition of cyclohexene hydroperoxide resulted in the formation of cyclohexenone as the only carbonyl identified (60% molar yield).
2. The yields of hexanal and 2,4-decadienal resulting from the decomposition of linoleic acid hydroperoxides varied from 5% to 12% for hexanal and 0% to 7% for 2,4-decadienal, depending on the temperature of decomposition. Mild temperatures seem to favor the formation of hexanal and high temperatures, the formation of 2,4-decadienal indicating a selective decomposition of the hydroperoxides.
3. Metal ions accelerated the decomposition of hydroperoxides and their effects were very close to high temperature effects. Cu^{++} formed remarkably high yields of carbonyls, most of them unknown carbonyls.
4. Most antioxidants accelerated the decomposition of hydroperoxides but resulted in lower yields of carbonyls depending on the antioxidant. The efficiency of an antioxidant should then be judged not only on the basis of its effect on the accumulation of hydroperoxides but also on the basis of its effect on scission products.
5. Linolenate hydroperoxides gave a larger number of carbonyls than linoleate, but linolenate hydroperoxides gave a lower total carbonyl yield than linoleate hydroperoxides.

REFERENCES

1. Badings, H. T. 1959. Isolation and identification of carbonyl compounds formed by autoxidation of ammonium linoleate. J. Am. Oil Chemists' Soc. 36:648-650.

2. Badings, H. T. 1960. Principles of autoxidation process in lipids with special regard to the development of autoxidation off-flavors. Neth. Milk Dairy J. 14:215-242.

3. Barlett, P. D., and A. A. Frimer. 1978. The reaction of singlet oxygen with 1-methoxycyclohexene. Heterocycles 11:419-434.

4. Bateman, L., and H. Hughes. 1952. The thermal decomposition of cyclohexenyl hydroperoxide in hydrocarbon solvents. J. Chem. Soc. 1952:4594-4601.

5. Bell, E. R., J. H. Raley, F. F. Rust, F. H. Sembold, and W. E. Vaughan. 1951. Reactions of free radicals associated with low temperature oxidation of paraffins. Disc. of the Faraday Soc. 10:242-249.

6. Bergström, S. 1945. On the autoxidation of methyl ester of linoleic acid. Mineral Geol. 21A, No. 14.

7. Bolland, J. L. 1949. Kinetics of olefin oxidation. Q. Rev. 3:1-21.

8. Bolland, J. L., and G. Gee. 1946. Kinetic studies in the chemistry of rubber and related materials. II. The kinetics of oxidation of conjugated olefins. Trans. Faraday Soc. 42:236-244.

9. Bolland, J. L., and H. P. Koch. 1945. The course of autoxidation reactions in polyisoprenes and related compounds. Part IX. The primary thermal oxidation product of ethyl linoleate. J. Chem. Soc. 1945.

10. Chan, H. W.-S. 1977. Photosensitized oxidation of unsaturated fatty acid methyl esters. The identification of different pathways. J. Am. Oil Chemists' Soc. 54:100-104.

11. Chan, H. W.-S., and G. Levett. 1977. Autoxidation of methyl linoleate: Separation and analysis of isomeric mixture of linoleate hydroperoxides and methyl hydroxylinoleate. Lipids 12:99-104.

12. Chan, H. W.-S., and G. Levett. 1977. Autoxidation of methyl linoleate: Analysis of methyl hydroxylinolenate isomers by HPLC. Lipids 12:837-840.

13. Chan, H. W.-S., C. T. Costaras, F. A. A. Prescott, and P. A. T. Swoboda. 1975. Specificity of lipoxygenoses. Thermal isomerizations of linoleate hydroperoxides, a phenomenon affecting the determination of isomeric ratios. Biochim. Biophys. Acta 398: 347-350.

14. Chan, H. W.-S., F. A. A. Prescott, and P. A. T. Swoboda. 1976. Thermal decomposition of individual positional isomers of methyl linoleate hydroperoxide: Evidence of carbon-oxygen bond scission. Lipids 53:572-576.

15. Chan, H. W.-S., G. Levett, and J. A. Matthew. 1979. The mechanism of the rearrangement of linoleate hydroperoxides. Chem. Phys. Lipids 24:245-256.

16. Cobern, D., J. S. Hobbs, R. A. Lucas, and D. J. MacKenzie. 1966. Location of hydroperoxide groups in monohydroperoxides formed by chlorophyll-photosensitized oxidation of unsaturated esters. J. Chem. Soc. (C) 1897-1902.

17. Delov, A. W., W. K. Rohwedder, and H. Dutton. 1967. Mechanism of lipoxidase reaction. 1. Specificity of hydroperoxidation of linoleic acid. Lipids 2:28-32.

18. Dixon, M. D., and E. G. Hammond. 1984. The flavor intensity of some carbonyl compounds important in oxidized fats. J. Am. Oil Chemists' Soc. 61:1452-1456.

19. Ellis, R., A. M. Gaddis, and G. T. Currie. 1961. Carbonyls in oxidizing fat. IV. The role of various fatty acid components in carbonyl generation. J. Food Sci. 26:131-138.

20. Farmer, E. H. 1942. α-methylene reactivity in olefinic and polyolefinic systems. Trans. Faraday Soc. 38:340-356.

21. Farmer, E. H., and A. Sundralingam. 1942. The course of autoxidation reaction in polyisoprenes and allied compounds. Part I. The structure and reactive tendencies of the peroxides of simple olefins. J. Chem. Soc. 1942:121-239.

22. Farmer, E. H., and D. A. Sutton. 1943. The course of autoxidation reactions in polyisoprenes and allied compounds. Part IV. The isolation and constitution of photo-chemically-formed methyl oleate peroxide. J. Chem. Soc. 1943:119-122.

23. Farmer, E. H., H. P. Koch, and D. A. Sutton. 1943. The course of autoxidation in polyisoprenes and allied compounds. Part VII. Rearrangement of double bonds during autoxidation. J. Chem. Soc. 1943:541-547.

24. Frankel, E. N. 1962. Hydroperoxides. Pp. 51-78. In M. W. Schultz and E. A. Day (eds.) Symposium on foods: Lipids and their oxidation. AVI Publishing Co., Inc., Westport, CT.

25. Frankel, E. N. 1979. Autoxidation. Pp. 353-378. In E. H. Pryde (ed.) Fatty acids. The Am. Oil Chemists' Soc., Champaign, IL.

26. Frankel, E. N. 1980. Lipid oxidation. Prog. Lipid Res. 19:1-22.

27. Frankel, E. N. 1984. Lipid oxidation: Mechanisms, products and biological significance. J. Am. Oil Chemists' Soc. 61:1908-1917.

28. Frankel, E. N., W. E. Neff, and T. R. Bessler. 1977. Analysis of autoxidized fats by gas chromatography-mass spectrometry. V. Photosensitized oxidation. Lipids 14:961-967.

29. Frankel, E. N., W. E. Neff, W. K. Rohwedder, B. P. S. Khambay, R. F. Garwood, and B. C. L. Weedon. 1977. Analysis of autoxidized fats by gas chromatography-mass spectrometry. I. Methyl oleate. Lipids 12:901-907.

30. Frankel, E. N., W. E. Neff, W. K. Rohwedder, B. P. S. Khambay, R. F. Garwood, and B. C. L. Weedon. 1977. Analysis of autoxidized fats by gas chromatography-mass spectrometry. II. Methyl linoleate. Lipids 12:908-913.

31. Frankel, E. N., W. E. Neff, W. K. Rohwedder, B. P. S. Khambay, R. F. Garwood, and B. C. L. Weedon. 1977. Analysis of autoxidized fats by gas chromatography-mass spectrometry. III. Methyl linolenate. Lipids 12:1055-1061.

32. Frankel, E. N., W. E. Neff, and E. Selke. 1981. Analysis of autoxidized fats by gas chromatography-mass spectrometry. VII. Volatile thermal decomposition products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate, and linolenate. Lipids 16:279-285.

33. Frankel, E. N., W. E. Neff, and E. Selke. 1983. Analysis of autoxidized fats by gas chromatography mass spectrometry. VIII. Volatile thermal decomposition products of hydroperoxy cyclic peroxides. Lipids 18:353-357.

34. Frankel, E. N., W. E. Neff, and E. Selke. 1984. Analysis of autoxidized fats by gas chromatography-mass spectrometry. IX. Homolytic vs. heterolytic cleavage of primary and secondary oxidation products. Lipids 19:790-800.

35. Frimer, A. A. 1979. The reaction of singlet oxygen with olefins: The question of mechanism. Chem. Rev. 79:359-387.

36. Gaddis, A. M., R. Ellis, and G. T. Currie. 1961. Carbonyls in oxidizing fat. V. The composition of neutral volatile monocarbonyl compounds from autoxidized oleate, linoleate, linolenate esters and fats. J. Am. Oil Chemists' Soc. 38:371-375.

37. Galliard, T., and D. R. Phillips. 1971. Lipoxygenase from potato tubers. Biochem. J. 124:431-438.

38. Gardner, H. W. 1975. Isolation of a pure isomer of linoleic acid hydroperoxide. Lipids 10:248-252.

39. Gardner, H. W., K. Eskins, G. W. Grams, and G. E. Inglett. 1972. Radical addition of linoleic acid hydroperoxides to α-tocopherol or the analogous hydroxychroman. Lipids 7:324-334.

40. Gardner, H. W., and E. Selke. 1984. Volatiles from thermal decomposition of isomeric methyl (12S,13S)-(E)-12,13-epoxy-9-hydroperoxy-10-octadecanoates. Lipids 19:375-380.

41. Grosh, W. 1976. Breakdown of linoleic acid hydroperoxides. Formation of volatile carbonyl compounds. Z. Lebensm.-Unters.-Forsch. 160:371-375.

42. Grosh, W. 1977. Breakdown of linoleic acid hydroperoxide in the presence of ascorbic acid. Analysis of the volatile aldehydes. Z. Lebensm.-Unters.-Forsch. 163:4-7.

43. Gunstone, F. D. 1984. Reaction of oxygen and unsaturated fatty acids. J. Am. Oil Chemists' Soc. 61:441-447.

44. Gunstone, F. D., E. G. Hammond, H. Schuler, G. M. Scrimgeour, and H. S. Vadanoyagam. 1975. Fatty acids. Part 44: The preparation of some long-chain hydroperoxides and t-butyl peroxides. Chem. Phys. Lipids 14:81-86.

45. Hall, G. E., and D. G. Roberts. 1966. A study by infrared and proton magnetic resonance spectroscopy of the monohydroperoxides of oleate, linoleate esters. J. Chem. Soc. 1966:1109-1112.

46. Hamberg, M., and B. Samuelsson. 1965. On the specificity of the lipoxidase catalyzed oxygenation of unsaturated fatty acids. Biochem. Biophys. Res. Commun. 21:531.

47. Hamberg, M., and B. Samuelsson. 1967. On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxidase. J. Biol. Chem. 242:5329-5335.

48. Hamm, D. L., E. G. Hammond, V. Parvanah, and H. E. Snyder. 1965. The determination of peroxides by the stamm method. J. Am. Oil Chemists' Soc. 42:920-922.

49. Hammond, E. G. 1985. Stability of soybean oil to oxidation. Pp. 251-258. In Richard Shibles (ed.) Proceedings of World Soybean Conference III. Westview Press, Boulder, CO.

50. Hammond, E. G., and F. D. Hill. 1964. The oxidized-metallic and grassy flavor components of autoxidized milk fat. J. Am. Oil Chemists' Soc. 41:180-183.

51. Haslbeck, F., and W. Grosh. 1983. Autoxidation of phenyl linoleate and phenyl oleate: HPLC analysis of the major and minor monohydroperoxides. Lipids 18:706-713.

52. Hiatt, R. 1971. Hydroperoxides. Pp. 1-152. In D. Swern (ed.) Organic peroxides. Vol. II. Wiley-Interscience. New York, NY.

53. Hiatt, R., T. Mill, and F. R. Mayo. 1968. Homolytic decomposition of hydroperoxides. I. Summary and implications for autoxidation. J. Org. Chem. 33:1416-1420.

54. Hill, L. M., E. G. Hammond, and R. G. Seals. 1969. Effect of antioxidants and synergists on peroxide decomposition in milk fat. J. Dairy Sci. 52:1914-1916.

55. Hock, H., and O. Schrader. 1936. On cyclohexene peroxide. Naturwissenschaften 24:159.

56. Ingold, K. U. 1962. Metal catalysis. Pp. 93-121. In H. W. Schultz, E. A. Day, and R. O. Sinnhuber (eds.) Lipids and their oxidation. AVI Publishing Co., Westport, CT.

57. Jensen, R. G., T. A. Marks, J. Sampugna, J. G. Quinn, and D. L. Carpenter. 1966. Purification of triglycerides with an alumina column. Lipids 1:451-452.

58. Johnson, D. C., and E. G. Hammond. 1971. A sensitive method for the determination of carbonyl compounds. J. Am. Oil Chemists' Soc. 48:653-656.

59. Kallio, H., R. R. Linko, and J. Kaitaranta. 1972. Gas-liquid chromatographic analysis of 2,4-dinitrophenylhydrazones of carbonyl compounds. J. Chromatography 65:355-360.

60. Kawahara, F. K., H. J. Dutton, and J. C. Cowan. 1952. Volatile cleavage products of autoxidized methyl linolenate. J. Am. Oil Chemists' Soc. 29:633-635.

61. Keeney, M. 1962. Secondary degradation products. Pp. 79-89. In H. W. Schultz and E. A. Day (eds.) Symposium on foods: Lipids and their oxidation. AVI Publishing Co., Inc., Westport, CT.

62. Khan, N. A., W. O. Lundberg, and R. T. Holman. 1954. Displacement analysis of lipids. IX. Products of the oxidation of methyl linoleate. J. Am. Oil Chemists' Soc. 76:1179-1784.

63. Kharash, M. S., and J. G. Burt. 1951. The chemistry of hydroperoxides. VIII. The acid-catalyzed decomposition of certain hydroperoxides. J. Org. Chem. 16:150-154.

64. Kimoto, W. I., and A. M. Gaddis. 1969. Precursors of alK-2,4-dienals in autoxidized lard. J. Am. Oil Chemists' Soc. 46:403-408.

65. Kimoto, W. I., and A. M. Gaddis. 1974. Monocarbonyl compounds from catalytic decomposition of autoxidized unsaturated fatty acid esters. J. Am. Oil Chemists' Soc. 51:307-311.

66. Lau, F. Y., and E. G. Hammond. 1982. Effects of randomization on the oxidation of corn oil. J. Am. Oil Chemists' Soc. 59:407-411.

67. Linko, R. R., H. Kallio, and K. Rainio. 1978. Gas-liquid chromatographic analysis of 2,4-dinitrophenylhydrazones of monocarbonyl compounds in carrots using glass capillary columns. J. Chromatogr. 155:191-194.

68. Loury, M. 1972. Possible mechanisms of autoxidative rancidity. Lipids 7:671-675.

69. Lundberg, W. O. 1961. Autoxidation and antioxidants. Vol. I. Interscience Publishers, New York, NY.

70. Lundberg, W. O. 1962. Autoxidation and antioxidants. Vol. II. Interscience Publishers, New York, NY.

71. Lundberg, W. O. 1962. Mechanisms. Pp. 31-50. In H. W. Schultz, E. A. Day, and R. O. Sinnhuber (eds.) Lipids and their oxidation. AVI Publishing Co., Westport, CT.

72. Lundberg, W. O., J. R. Chipault, and M. J. Hendrickson. 1949. Observations on the mechanism of the autoxidation of methyl linoleate. J. Am. Oil Chemists' Soc. 26:109-115.

73. Michalski, S. T., and E. G. Hammond. 1972. Use of labeled compounds to study the mechanism of flavor formation in oxidizing fats. J. Am. Oil Chemists' Soc. 49:563-566.

74. Neff, W. E., and E. N. Frankel. 1980. Quantitative analyses of hydroxystearate isomers from hydroperoxides by HPLC of autoxidized and photosensitized-oxidized fatty esters. Lipids 15:587-590.

75. Neff, W. E., E. N. Frankel, and D. Weislender. 1982. Photosensitized oxidation of methyl linolenate. Secondary products. Lipids 17:780-790.

76. Neff, W. E., E. N. Frankel, E. Selke, and D. Weislender. 1983. Photosensitized oxidation of methyl linoleate monohydroperoxides: Hydroperoxy cyclic peroxides, dihydroperoxides, keto esters, and volatile thermal decomposition products. Lipids 18:868-876.

77. Pappo, R., D. S. Allen, Jr., R. U. Lemieux, and W. S. Johnson. 1956. Osmium tetroxide-catalyzed periodate oxidation of olefinic bonds. J. Org. Chem. 21:478-479.

78. Paquette, G., D. B. Kupranycz, and F. R. van de Voort. 1985. The mechanisms of lipid autoxidation. I. Primary oxidation products. Can. Inst. Food Sci. Technol. J. 18:112-118.

79. Peers, K. E., D. T. Coxon, and H. W.-S. Chan. 1984. Thermal decomposition of individual positional isomers of methyl linolenate hydroperoxides, hydroperoxy cyclic peroxides and dihydroperoxides. Lipids 19:307-313.

80. Piretti, M. V., P. Capella, and G. Bonaga. 1974. Geometrical and positional isomers of oleic acid hydroperoxides. J. Chromatogr. 92:196-200.

81. Privett, O. S., and F. W. Quakenbush. 1954. Effects of antioxidants on the thermal decomposition of fat peroxides in vacuo. J. Am. Oil Chemists' Soc. 31:281-283.

82. Ross, J., A. I. Gebhart, and J. F. Gerecht. 1949. The autoxidation of methyl oleate. J. Am. Chemists' Soc. 71:282-286.

83. Seals, R. G., and E. G. Hammond. 1969. Some carbonyl flavor compounds of oxidized soybean and linseed oils. J. Am. Oil Chemists' Soc. 47:278-280.

84. Selke, E., E. N. Frankel, and W. E. Neff. 1978. Thermal decomposition of methyl oleate hydroperoxides and identification of volatile components by gas chromatography-mass spectrometry. Lipids 13:511-513.

85. Selke, E., W. K. Rohwedder, and H. J. Dutton. 1980. Volatile components from trilinolein heated in air. J. Am. Oil Chemists' Soc. 57:25-29.

86. Sephton, H. H., and D. A. Sutton. 1956. The chemistry of polymerized oils. V. The autoxidation of methyl linoleate. J. Am. Oil Chemists' Soc. 33:263-272.

87. Schwartz, D. P., H. S. Haller, and M. Keeney. 1963. Direct quantitative isolation of monocarbonyl compounds from fats and oils. Anal. Chem. 35:2191-2194.

88. Sherwin, E. R. 1976. Antioxidants for vegetable oils. J. Am. Oil Chemists' Soc. 53:430-436.

89. Stephens, H. N. 1928. Studies in auto-oxidation. I. Cyclohexene peroxide. J. Am. Chemists' Soc. 50:568-571.

90. Stuckey, B. N. 1962. Antioxidants. Pp. 139-150. In H. W. Schultz, E. A. Day, and R. O. Sinnhuber (eds.) Lipids and their oxidation. AVI Publishing Co., Westport, CT.

91. Swift, C. E., F. G. Dollear, E. G. Lawrence, and R. T. O'Connor. 1948. Decomposition of methyl hydroperoxide oleate. J. Am. Oil Chemists' Soc. 25:39-40.

92. Swift, C. E., R. T. O'Connor, L. E. Brown, and F. G. Dollear. 1949. The aldehydes produced during the autoxidation of cottonseed oil. J. Am. Oil Chemists' Soc. 26:297-300.

93. Swoboda, B. A. T., and C. H. Lea. 1965. The flavor volatiles of fats and fat-containing foods. II. A gas chromatographic investigation of volatile autoxidation products from sunflower oil. J. Sci. Food Agric. 16:680-689.

94. Terao, J., and S. Matsuchita. 1977. Products formed by photosensitized oxidation of unsaturated fatty acid esters. J. Am. Oil Chemists' Soc. 54:234-238.

95. Tripp, R. C., T. Richardson, C. H. Amundson, and J. H. von Elbe. 1969. The gas-liquid chromatography of nitro and chloro substituted phenylhydrazones of n-alkanals and n-methyl ketones and syn-anti-isomers. J. Am. Oil Chemists' Soc. 46:134.

96. Veldink, G. A., J. F. G. Vliegenthart, and J. Boldingh. 1977. Plant lipoxygenases. Prog. Chem. Fats Other Lipids 15:131-166.

97. White, P. J., and E. G. Hammond. 1983. Quantification of carbonyl compounds in oxidized fats as trichlorophenylhydrazones. J. Am. Oil Chemists' Soc. 60:1769-1773.

98. Ziegler, K., A. Spath, E. Schaaf, W. Schumann, and E. Winkelmann. 1942. The halogenation of unsaturated substances in the allylic position. Liebigs Ann. Chem. 551:110.

99. Zimmerman, D. C., and B.A. Vick. 1970. Specificity of flaxseed lipoxidase. Lipids 5:392-397.

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